Med Chem Res (2013) 22:3802–3811
DOI 10.1007/s00044-012-0382-6

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Docking, synthesis, and pharmacological investigation of novel substituted thiazole derivatives as non-carboxylic, anti-inflammatory, and analgesic agents

S. N. Thore · Sunil V. Gupta · Kamalkishor G. Baheti

Received: 15 May 2012 / Accepted: 27 November 2012 / Published online: 11 December 2012

Abstract A series of substituted thiazole derivatives (**6–16**) were synthesized to obtain new compounds with potential anti-inflammatory and analgesic activities. At equimolar oral doses, compounds 3-(piperidin-1-yl-methyl)-1, 3, 4-oxadiazol-2-thione (**14**), 5-amino-4-ethyl ester pyrazole (**15**), and 5-amino-3-phenylpyrazole derivatives (**16**) displayed anti-inflammatory and analgesic activities significant to those of diclofenac sodium in the carrageenan-induced paw edema test in rat and acetic acid-induced writhing test in mice,-respectively. The most active members of the series (**9, 11, 14, 15**, and **16**) were selected for ulcerogenic potential study. These compounds exhibited quite less ulcerogenic index in the range of 0.44 to 0.62 whereas diclofenac sodium showed 4.67. The docking study results also indicated that the compound **6, 7, 8, 11,** and **14** exhibited the docking score ranging from −3.951 to −4.691.

Keywords Thiazole · Anti-inflammatory · Analgesic · Ulcerogenicity

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. NSAIDs act by suppressing the prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endo peroxidase, which is also known as cyclo-oxygenase (COX) (Palomer *et al*., 2002). The enzyme COX exists in two isoforms viz. COX-1 and COX-2, which is regulated and expressed differently (Dannhardt and Kiefer 2001). COX-1 provides cytoprotection in the gastrointestinal tract (GIT) whereas inducible COX-2 is responsible for inflammation. Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) act via the inhibition of the COX-1 isoenzyme or the combined inhibition of COX-1 and COX-2 isoenzymes. The COX-1 is mainly responsible for mucus formation in the gastrointestinal (GI) tract, COX-1 inhibition is blamed for inducing GI irritation, bleeding, and ulceration which are main undesired side effects of such drugs (Leval *et al*., 2002). The COX-2 isoenzyme was found to be over expressed during inflammation hence, drug investigation was focused on selective COX-2 inhibition, in view to prevent inflammation by side stepping the undesired side effect of COX-1 inhibitors (Jouzeau *et al.,* 1997; Fu *et al*., 1990). Therefore, selective COX-2 inhibitors with better safety profile have been marketed as a new generation NSAIDs (Tally *et al*., 2000). Most of the clinical NSAIDs possess acidic carboxyl (COOH) group, which further causes GI irritation by direct contact of carboxyl 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. Hence, the discovery and development of novel anti-inflammatory and analgesic agents with safety profile is still a necessity. Several studies have described that the derivatization of the carboxylate function of NSAID with various non-acidic or less-acidic azoles, viz. 1, 3, 4-oxadiazole, triazole, tetrazole (Shashikant *et al.,* 2008; Amit *et al.,* 2000), etc. which resulted in an improved anti-inflammatory activity with reduced ulcerogenicity. Various non-acidic (Amir *et al*.,

S. N. Thore (✉) · S. V. Gupta
Department of Chemistry, Vinayakrao Patil Mahavidyalaya, Vaijapur 423 701, Aurangabad, India
e-mail: snthore@rediffmail.com

K. G. Baheti
Charak College of Pharmacy and Research, Wagholi 412 207, Pune, India

2007) and acid bioester of the type of 1, 3, 4-thiadiazole and 1, 3, 4-oxadiazole derivatives (Diane *et al*., 1993; Manjunathaa *et al*., 2010) were reported for anti-inflammatory activity. Owing to increased hydrolytic (Clapp 1976) and metabolic stabilities of oxadiazoles, oxadiazolinone, oxadiazolinethione, and tetrazole ring, improved pharmacokinetic and in vivo performance is often observed, which makes these heterocycles as an important structural motif for the pharmaceutical research. Thiazoles are the important biological active compounds present in sulfathiazole (antimicrobials), ritonaveir (antiretroviral), and triazofurin (antineoplastic). Thiazole derivatives have also been reported for their anti-inflammatory activity (Sharma *et al*., 2009). In one study, it was reported that the phenyl thiazole derivatives (Fentiazac) were acting through selectively inhibiting COX-2 enzyme (Brown *et al.,* 1974). Fentiazac is chemically 2-[4-(4-chlorophenyl)-2-phenyl-1,3-thiazol-5-yl]acetic acid and was marketed for relieving the muscular pain and inflammation. In our attempt to synthesize safer and potent agents for the treatment of inflammatory diseases, we have replaced carboxylic acid group present in thiazole of Fentiazac with non-acidic or less-acidic heterocycles to accentuate potency and to reduce GI toxicities associated with traditional NSAIDs due to its free –COOH group.

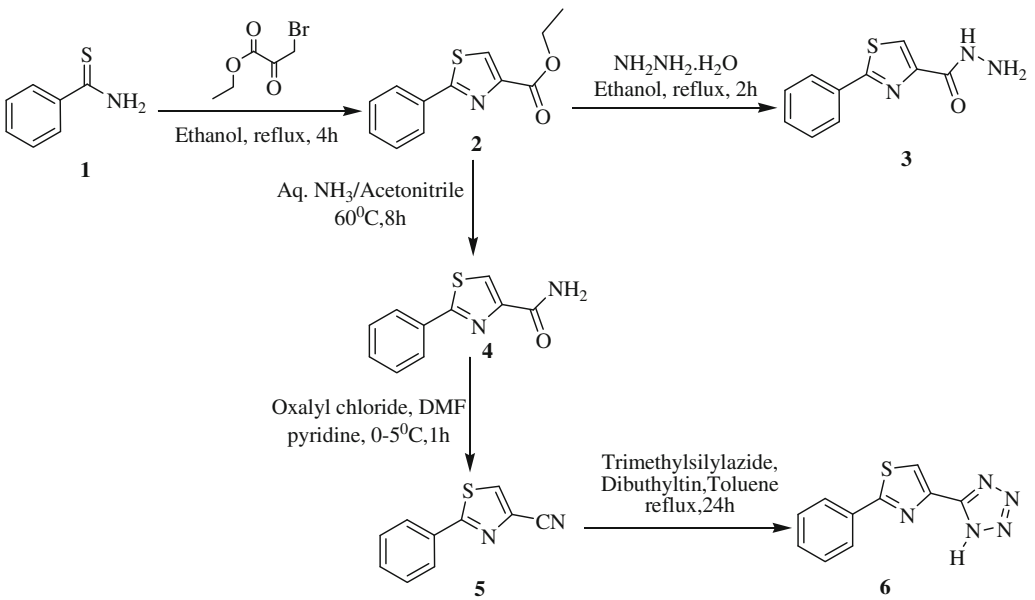
The present paper describes the docking, synthesis, and evaluation of novel substituted thiazole derivatives for anti-inflammatory, analgesic, and ulcerogenic activities. These derivatives were substituted by heterocycles like 1, 3, 4-oxadiazole, 1, 3, 4-oxadiazolinone 1, 3, 4-oxadiazolinethione, tetrazole, and pyrazole moiety at position 4 of thiazole. The thiazole ring is selected as a part of designed molecule because of its less-acidic characteristic and its biological importance. The less-acidic characteristic of thiazole will be useful in reducing the GI toxicity which is associated with traditional NSID due to free carboxyl group.

Result and discussion

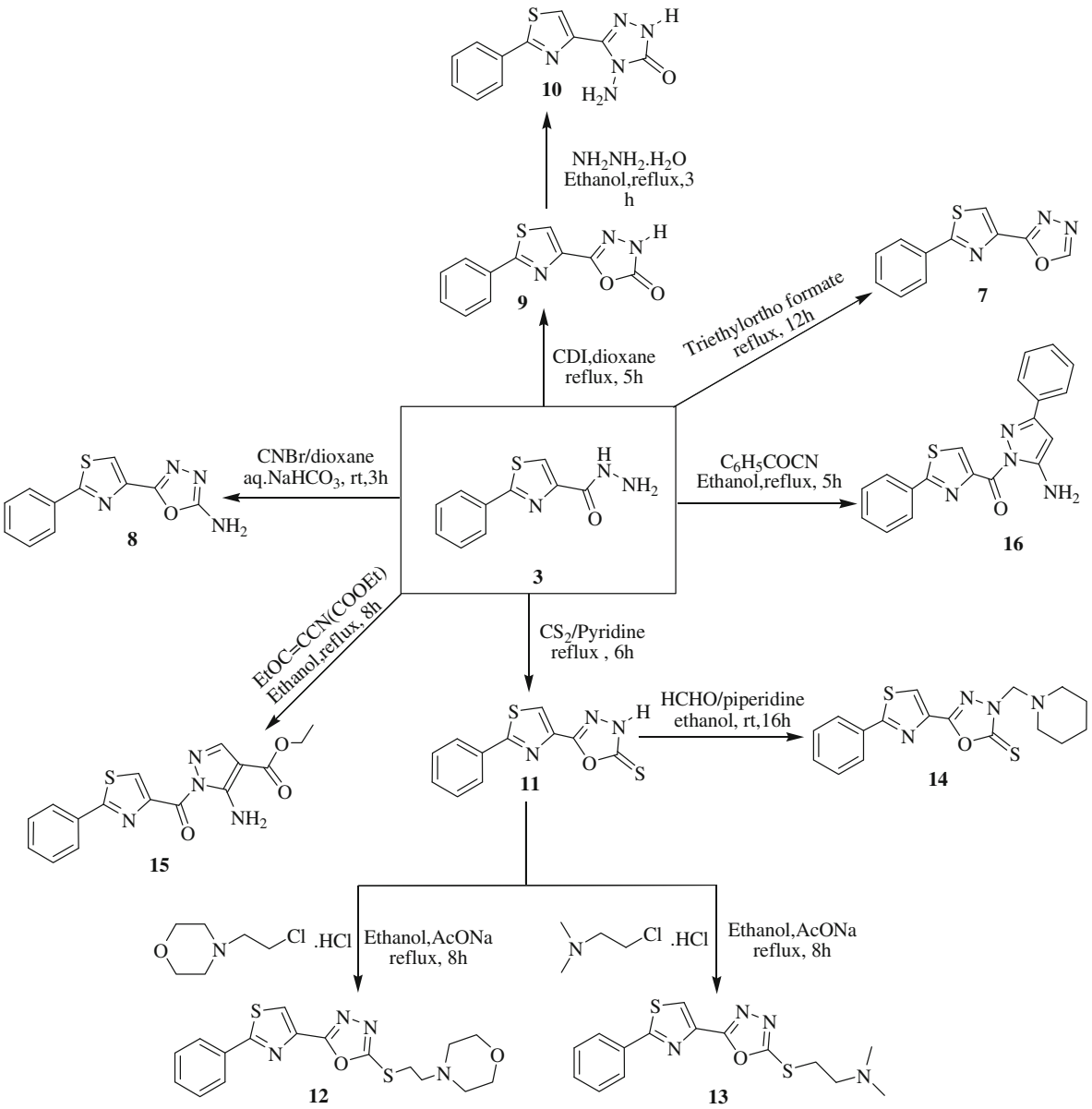
Synthesis of the title compounds (**6-16**) is outlined in (Schemes 1 and 2). The parent compound ethyl-2-phenylthiazole-4-carboxylate (**2**) (Hall and James 1966) was synthesized by reaction of thio benzamide (**1**) with ethyl bromopyruate in ethanol. The compound (**2**) on reflux with conc. ammonia solution in the acetonitrile led to the formation of 2-phenylthiazole-4-carboxamide **(4)**, which on further treatment with oxalyl chloride in DMF afforded 2-phenyloxazole-4-carbonitrile (**5**). The compound (**5**) possesses cyano group and can be conveniently converted to tetrazole derivative **(6)** by treatment with trimethyl silyl azide in the presence of dibutyl tin at reflux temperature.

The compound (**2**) on treatment with hydrazine hydrate afforded 2-phenylthiazole-4-carboxhydrazide (**3**) (Scheme 1). 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 triethyl orthoformate. The 2-amino-l, 3, 4-oxadiazoles (**8**) was obtained from the action of cyanogen 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. 1, 3, 4-oxadiazol-2-one (**9**);



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



Scheme 2 Synthesis of target compounds **7–16**

when treated with hydrazine hydrate in boiling ethanol, it afforded the corresponding aminotriazolone (**10**).

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 **(11)**. Alkylation of 1,3,4-oxadiazol-2-thione **(11)** with alkylating agents such as 4-(2-chloroethyl)morpholinyl hydrochloride and 2-dimethyaminoethyl chloride hydrochloride in boiling ethanol in the presence of fused sodium acetate independently gave required compounds which have been assigned the structures (**12)** and **(13),** respectively. The resultant 1, 3, 4-oxadiazol-2-thiones **(11)** were further converted into corresponding mannich bases **(14)** on aminomethylation with formaldehyde and piperidine.

Finally, acid hydrazide **(3)** on reaction with ethyl (ethoxy methylene) cyanoacetate in ethanol gave the corresponding 5-amino-4-ethylester pyrazole derivatives **(15).** The 5-amino-3-phenylpyrazole derivative **(16)** 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 has been reported in experimental protocols. The IR spectral characteristics of compounds **(6-16)** showed absorption bands in the ranges of 3150–3430 cm^{-1} for NH_2, 3000–3040 cm^{-1} for Ar–H,1610–1640 $cm^{-1}$for C = N, and1320–1300 cm^{-1} for C = S. For 1, 3, 4-oxadiazole derivatives (**7–9** and **11–14**), the presence of C = N stretching band at 1610–1640 cm^{-1} is an evidence of ring closure. Oxadiazole **(9, 10**

and **11)** showed N–H stretching bands at 3150–3310 cm^{-1}. The absence of absorptions of the amide carbonyl in the region 1675 cm^{-1} and hydrazide in the region 3380–3125 cm^{-1} indicated formation of azoles **(6–16)** from the corresponding acid amide **(4)** and acid hydrazide **(3)**.

^{1}H NMR spectrum of compound **(2)** showed triplet at δ 1.29–1.34 due to CH_3 of CH_2-CH_3, and quartrate peak appeared at δ 4.2–4.32 due to CH_2 of CH_2-CH_3 at position 4. The ^{1}H NMR spectra of compound **(6-16)** showed singlet at δ 7.77–8.50 due to thiazole proton, and a multiplet at δ 7.34–8.10 was observed for aromatic protons. For compound (**3**), a broad singlet peak at δ 4.49 for NH_2 and δ 8.70 for NH was observed. All other derivatives exhibited satisfactory chemical shifts which confirmed the assigned structures of compounds.

The results of docking study were predicted in by docking score and are given in the (Table 1). More negative score indicates the better binding with selected receptor. The docking scores of designed target molecules were compared with marketed COX-2 inhibitors, indomethacine and celecoxib. The designed molecules **6**, **7**, **8, 11,** and **14** exhibited the docking scores of −4.088, −4.105, −4.691, −4.629, and −3.951,-respectively, whereas standard indomethacine showed a docking score of -4.559. The compound **14** showed good interactions with different amino acids of the receptor-like PHE 470C and ARG 120C (Fig. 1) whereas standard indomethacine exhibited interactions with ARG 120C (Fig. 2).

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 2 h. Decline in activity was observed at 3 h. All the compounds in the series (**6–16**) exhibited the anti-inflammatory activity in the range of 14–45 % after 1 h and 21–60 % after 3 h. The compounds **14** and **15** exhibited 44 and 45 % anti-inflammatory activities, -respectively, whereas standard diclofenac sodium showed 40 % activity at 1 h of study. The same compounds showed 55 and 60 % activities at 3 h, whereas standard diclofenac sodium exhibited 55 % activity at 3 h. The structural correlation with anti-inflammatory activity showed that compounds with 1, 3, 4-oxadiazole-2(3H)-one, 1, 3, 4-oxadiazole-2(3H)-thione, and pyrazole moiety showed better activity. All the compounds(**9,11,14,15,** and **16**) possessing these ring moieties exhibited activities in the range of 48–60 % after 2 and 3 h of studies.

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 activities in the range of 16–61 % (Table 2). Among the derivatives, the compounds (**11, 14, 15,** and **16)** showed analgesic activities around 58–61 %

Table 1 Results of docking scores and anti-inflammatory activities of title compounds (**6–16**) against carrageenan-induced rat paw edema model in rats

Compound	Change in paw volume in (ml) after drug treatment (±SEM)			Anti-inflammatory activity (% inhibition)			Dock score
	1 h	2 h	3 h	1 h	2 h	3 h	
Control	0.900 ± 0.03**	1.110 ± 0.07**	1.200 ± 0.08**	–	–	–	–
Standard	0.762 ± 0.04**	0.800 ± 0.09**	0.850 ± 0.04**	40.60	56.66	54.92	–
6	0.825 ± 0.02**	0.885 ± 0.08**	0.935 ± 0.01**	30.84	38.07	40.41	−4.088
7	0.842 ± 0.06**	0.962 ± 0.07**	1.052 ± 0.09**	24.24	31.48	26.98	−4.105
8	0.880 ± 0.08**	1.012 ± 0.07**	1.105 ± 0.09**	18.18	25.50	21.42	−4.691
9	0.825 ± 0.02**	0.885 ± 0.08**	0.935 ± 0.01**	34.84	49.07	48.41	−2.602
10	0.844 ± 0.04**	0.979 ± 0.06**	1.074 ± 0.09**	15.15	23.14	19.04	−3.857
11	0.735 ± 0.04**	0.780 ± 0.06**	0.840 ± 0.04**	37.87	53.78	50.79	−4.062
12	0.864 ± 0.01**	0.995 ± 0.08**	1.076 ± 0.08**	14.54	23.51	21.58	−2.075
13	0.787 ± 0.05**	0.904 ± 0.07**	0.999 ± 0.04**	22.72	31.11	25.87	−3.275
14	0.764 ± 0.04**	0.812 ± 0.09**	0.862 ± 0.07**	44.84	57.40	55.55	−3.951
15	0.721 ± 0.04**	0.740 ± 0.08**	0.790 ± 0.05*	45.15	62.96	60.31	−2.952
16	0.851 ± 0.08**	0.873 ± 0.07**	0.942 ± 0.02**	42.12	60.55	55.23	−3.046
Indomethacin							−4.559

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

* $p < 0.05$, ** $p < 0.01$ significant from control

Dose levels: test compounds and diclofenac sodium (25 mg/kg, b.w.p.o.)

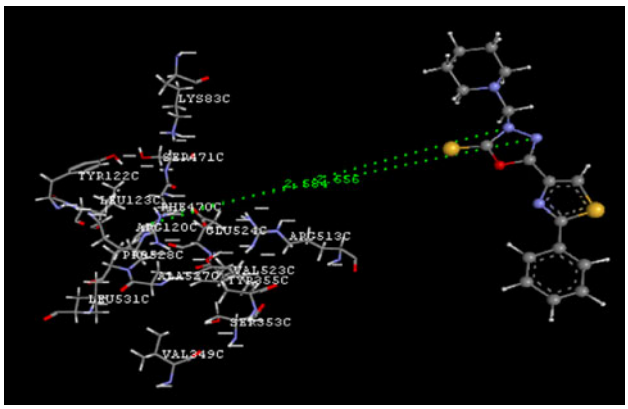


Fig. 1 H-bond interaction of compound 14 with amino acid PHE 470C and ARG 120C shown in *green color* (Color figure online)

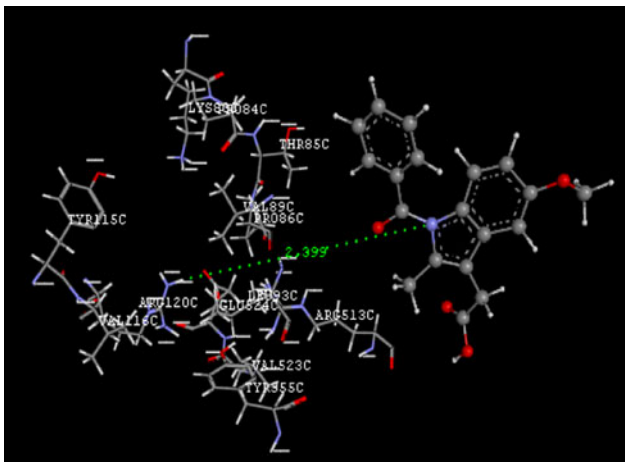


Fig. 2 H-bond interaction of Indomethacin with amino acid ARG 120C shown in *green color* (Color figure online)

whereas standard diclofenac sodium exhibited activity 58 %. The compound **9** showed moderate activity (42.22 %).

The compounds which showed significant anti-inflammatory and analgesic activities have been selected for acute ulcerogenicity studies. Ulcerogenic effects of substituted thiazole derivatives (**9, 11**, **14, 15,** and **16**) 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 4.67 ± 0.75), the test compounds exhibited 10–13 % of the ulcer index of the reference standards. The compounds (**9**, **11,** and **14**) exhibited the lowest ulcer indices of 0.44, 0.52, and 0.54-respectively, among the tested compounds. Incorporation of 1, 3, 4-oxadiazole-2(3H)-thione and pyrazole at position 4 produced the most-active and less ulcerogenic new chemical entities in the series.

Table 2 Analgesic activity of title compounds (**6–16**) against acetic acid-induced writhing tests in mice

Compound	No. of writhes in 10 min after treatment (mean ± SEM)	(%) inhibition
Control	31.33 ± 1.92**	–
Standard	13.16 ± 0.70**	57.99
6	26.16 ± 0.60**	16.50
7	25.66 ± 0.49**	18.09
8	24.96 ± 0.60**	20.33
9	18.10 ± 1.25**	42.22
10	25.16 ± 0.94**	19.69
11	13.13 ± 0.42*	58.09
12	24.50 ± 1.11**	21.80
13	25.50 ± 0.76**	18.60
14	12.93 ± 1.05**	58.72
15	12.16 ± 0.70*	61.18
16	12.50 ± 0.67**	60.10

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

* $p < 0.05$, ** $p < 0.01$ significant from control

Dose levels: test compounds & diclofenac sodium (25 mg/kg b.w. p.o)

Table 3 Evaluation of ulcerogenicity index

Compound	Dose mg/kg/day (p.o.)	Time (days)	Ulcer index
Control	CMC 1 %w/v	4	–
Standard	25	4	4.67 ± 0.75**
9	25	4	0.44 ± 0.42**
11	25	4	0.52 ± 0.50**
14	25	4	0.54 ± 0.73**
15	25	4	0.60 ± 0.38**
16	25	4	0.62 ± 0.43**

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

** $P < 0.01$ significant from control

Materials and methods

Chemistry

Melting points were taken in open capillary on a Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded on JASCO FTIR-4100 using KBr pellets. ^{1}HNMR spectra were recorded on Bruker 300-MHz FTNMR spectrometer in DMSO d_6 or $CDCl_3$ with Tetramethyl silane as an internal standard. Chemical shifts were reported in parts per million (δ ppm). Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB [positive]). Elemental analysis was

performed on a Perkin-Elmer 2400 C, H, and N analyzer. The progress of reaction was monitored on readymade silica gel plates (Merck) using chloroform–methanol (9:1) as a solvent system. Iodine was used as visualizing agent. Spectral data (IR, NMR, and mass spectra) confirmed the structures of the synthesized compounds, and the purity of these compounds was ascertained by microanalysis. Elemental (C, H, and N) analyses indicated that the calculated and observed values were within the acceptable limits (± 0.4 %). All chemicals used in the study were of reagent grade and were purified whenever necessary. All organic solvents were purified according to standard method.

Synthesis of ethyl-2-phenylthiazole-4-carboxylate ***(2)*** *(Hall and James 1966)*

Thio benzamide **1** (4.0 g, 29.1 mmol) and ethyl bromo pyruvate (6.26 g, 32.0 mmol) were dissolved in 40 ml ethanol. The reaction mixture was refluxed for 6 h. The excess of ethanol was removed by distillation to yield semisolid, which was purified by flash chromatography eluting with hexane–ethyl acetate (1:1) to provide yellow solid.

Yield = 5.0 g (74 %); m.p. 44–46 °C (45-47 °C lit.); IR (cm^{-1}): 3013 (w, Ar–CH), 1730 (s, C = O); ^1H NMR ($CDCl_3$, δ, ppm): 1.29–1.34 (t, 3H, J = 7.0 Hz, CH_3), 4.20-4.33 (q, 2H, J = 7.0 Hz, CH_2), 7.40-8.10 (m, 5H, Ar–H), 8.40 (s, 1H, thiazole-H); MS (*m/z*): 234 [M^++1].

Synthesis of 2-phenylthiazole-4-carbohydrazide ***(3)***

Ethyl-2-phenylthiazole-4-carboxylate **2** (2.5 g, 10.0 mmol) was dissolved in 10 ml ethanol in a conical flask; to this hydrazine, hydrate (1.61 g, 30.0 mmol) was added and stirred for 2 h at room temperature. The precipitate that appeared in the flask was filtered and recrystallized from ethanol to yield white crystalline product.

Yield = 2.0 g (85 %); m.p. 137–140 °C; IR (cm^{-1}): 3310,3245 (m, NH_2), 3160 (m, NH),1665 (s, C = O); ^1H NMR (DMSO-d_6,δ, ppm): 4.49 (bs, 2H, NH_2), 7.34–8.10 (m, 5H, Ar–H), 8.45 (s, 1H, thiazole-H), 8.70 (s, 1H, NH); MS(m/z): 220 [M^++1]. *Anal.* Calcd for: $C_{10}H_9N_3OS$: C, 54.78; H, 4.14; N, 19.16. Found: C, 54.72; H, 4.12; N, 19.14.

Synthesis of 2-phenylthiazole-4-carboxamide ***(4)***

Ethyl-2-phenylthiazole-4-carboxylate **2** (1.0 g, 4.29 mmol) was dissolved in 10 ml of acetonitrile. To this, conc. ammonia (10 ml) was added dropwise, and reaction mixture was heated on water bath at 50–60 °C for 8 h. The solid was separated by filtration and dried to obtain white crystals.

Yield = 0.7 g (80 %); m.p. 151–154 °C; IR (cm^{-1}): 3423, 3210 (m, NH_2), 3020 (w, Ar), 1675(s, C = O); ^1H NMR ($CDCl_3$, δ, ppm): 4.90 (bs, 2H, NH_2), 7.45-8.21 (m, 5H, Ar–H), 7.97 (s, 1H, thiazole-H); MS(m/z): 205 [M^++1]; *Anal.* Calcd for $C_{10}H_8N_2OS$: C, 58.81; H, 3.95; N, 13.72. Found: C, 58.78; H, 3.94; N, 13.70.

Synthesis of 2-phenylthiazole-4-carbonitrile ***(5)*** *(Swain 2007)*

To the solution of 2-phenylthiazole-4-carboxamide **4** (0.5 g, 2.45 mmol) in 10 ml dimethylformamide, oxalyl chloride (0.46 g, 3.67 mmol) was added, and the reaction mixture was stirred at 0–5 °C for 30 min. Then, pyridine (0.58 g, 7.35 mmol) was added and stirred again for 10 min. The reaction mixture was poured on ice water and extracted with dichloromethane. The organic layer was concentrated to obtain white crystals.

Yield = 0.4 g (89 %); m.p. 94–97 °C, (95–97 °C lit.); IR (cm^{-1}): 3030 (w, Ar–CH), 2239 (v, CN); ^1H NMR ($CDCl_3$, δ, ppm): 7.45–8.10 (m, 5H, Ar–H), 8.38 (s, 1H, thiazole-H); MS (m/z): 187 [M^++1]; *Anal.* Calcd for $C_{10}H_6N_2S$: C, 64.49; H, 3.25; N, 15.04. Found: C, 64.45; H, 3.24; N, 14.98.

Synthesis of 2-phenyl-4-(1H-tetrazol-5-yl) thiazole ***(6)***

To 2-Phenylthiazole-4-carbonitrile **5** (0.25 g, 1.34 mmol), trimethylsilyl azide (0.3 g, 2.68 mmol) and dibutyltin (20 mg) in 10 ml toluene were added; the reaction mixture was refluxed for 24 h. The excess of toluene was removed by distillation and extracted with ethyl acetate. The organic layer obtained was washed with water and concentrated to get white-colored compound.

Yield = 0.15 g (50 %); m.p. 167–170 °C; IR (cm^{-1}): 3310 (m, NH), 3030 (w, Ar–CH), 1640 (m, C = N); ^1H NMR ($CDCl_3$, δ, ppm): 7.34-8.01 (m, 5H, Ar–H), 8.10 (s, 1H, thiazole-H); MS(m/z): 230 [M^++1]; *Anal.* Calcd for $C_{10}H_7N_5S$: C, 52.39; H, 3.08; N, 30.08. Found: C, 52.35; H, 3.10; N, 30.0.

Synthesis of 2-(2-phenylthiazole-4-yl) [1, 3, 4] oxadiazole ***(7)***

A mixture of **3** (0.25 g, 1.1 mmol) and triethylorthoformate (5 ml) was refluxed for 12 h. The excess of the solvent was distilled off under reduced pressure, and the solid product obtained was filtered off and recrystallized from ethanol to yield white crystalline product.

Yield = 0.2 g (77 %); m.p. 190–193 °C; IR (cm^{-1}): 3030 (w, Ar–CH), 1610 (m, C = N); ^1H NMR (DMSO-d_6,δ, ppm): 7.34–8.01 (m, 5H, Ar–H), 8.19(s, 1H, thiazole-H), 8.38 (s, 1H, oxadiazole-H); MS(m/z): 230 [M^++1].

Anal. Calcd for: $C_{11}H_7N_3OS$: C, 57.63; H, 3.08; N, 18.33. Found: C, 57.60; H, 3.12; N, 18.21.

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

To sodium bicarbonate (0.96 g, 1.25 mmol) in 5-ml of water was added to a solution of **3** (0.25 g, 1.11 mmol) in 10 ml of dioxane at room-temperature and stirred for 5 min. To the stirred solution, cyanogens bromide (0.133 g, 1.25 mmol) was added slowly and it was kept for stirring for 3 h. The solid product that appeared was filtered and dried in vacuum. The solid was recrystallized from ethanol to afford brown-colored product.

Yield = 0.19 g (70 %); m.p. 201–204 °C; IR (cm^{-1}): 3380, 3124 (m, NH_2), 3020 (w, Ar–CH), 1615 (m, C = N); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.37 (bs, 2H, NH_2), 7.50–7.96 (m, 5H, Ar–H), 7.97 (s, 1H, thiazole-H); MS(m/z): 245 [M^++1]. *Anal.* Calcd for: $C_{11}H_8N_4OS$: C, 54.09; H, 3.30; N, 22.94. Found: C, 54.0; H, 3.32; N, 22.81.

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

A mixture of **3** (0.25 g, 1.11 mmol) and N, N'-carbonyldiimidazole (0.27 g, 1.71 mmol) in dioxane (10 ml) was heated under reflux for 5 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 = 0.22 g (81 %); m.p. 132–135 °C; IR (cm^{-1}): 3180 (m, NH), 3010 (w, Ar–CH), 1690 (s, C = O), 1622 (m, C = N); ^{1}H NMR (DMSO-d_6,δ, ppm): 7.34-8.12 (m, 5H, Ar–H), 8.17 (s, 1H, thiazole-H), 9.42 (s, 1H, NH); MS(m/z): 246 [M^++1]. *Anal.* Calcd for: $C_{11}H_7N_3O_2S$: C, 53.87; H, 2.88; N, 17.13. Found: C, 53.88; H, 2.82; N, 17.11.

Synthesis of 4-amino-5-(2-phenylthiazole-4-yl)-2,4-dihydro[1,2,4]triazole-3-one ***(10)***

A mixture of the oxadiazolinone **9** (0.3 g, 1.22 mmol) and hydrazine hydrate (2 ml) in ethanol (5 ml) was heated under reflux for 3 h. After cooling, the solvent was removed in vacuum, and the residue obtained was triturated with water. The solid was filtered and recrystallized from ethanol, which afforded the title compound **10** as white crystals.

Yield = 0.21 g (68 %); m.p. 178–181 °C; IR (cm^{-1}): 3253, 3180 (m, NH_2), 3156 (m, NH), 3030 (w, Ar–CH), 1690 (s, C = O), 1610 (m, C = N); ^{1}H NMR (DMSO-d_6,δ, ppm): 5.20 (bs, 2H, NH_2) 7.33-8.10 (m, 5H, Ar–H), 8.45 (s, 1H, thiazole-H), 10.45 (s, 1H, NH); MS(m/z): 260 [M^++1]. *Anal.* Calcd for: $C_{11}H_9N_5OS$: C, 50.96; H, 3.50; N, 27.01. Found: C, 50.88; H, 3.52; N, 27.0.

Synthesis of 5-(2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2(3H)-thione ***(11)***

A mixture of **3** (0.5 g, 2 mmol) and carbon disulfide (3 ml) in pyridine (10 ml) was refluxed on water bath for 6 h. After cooling, the solvent was evaporated under reduced pressure, and the residue was triturated with an ice–water mixture and neutralized with dilute hydrochloric acid. The separated solid product was filtered, washed with water, dried, and crystallized from ethanol afforded **11** as pale yellow crystals.

Yield = 0.45 g (76 %); m.p. 198–201 °C; IR (cm^{-1}): 3210 (m, NH), 3015 (w, Ar–CH), 1628 (m, C = N),1315 (m,C = S); ^{1}H NMR (DMSO-d_6,δ, ppm): 7.53-8.01 (m, 5H, Ar–H), 8.50 (s, 1H,thiazole-H), 10.55 (s, 1H, NH); MS(m/z): 262 [M^++1]. *Anal.* Calcd for: $C_{11}H_7N_3OS_2$: C, 50.56; H, 2.70; N, 16.08. Found: C, 50.55; H, 2.62; N, 16.0.

Synthesis of 4-{2-[5-(2-phenylthiazole-4-yl)-1,3,4-oxadiazole-2-ylthio]ethyl}-morpholine ***(12)***

A mixture of the oxadiazolinethione **11** (0.2 g, 0.72 mmol), fused sodium acetate (0.31 g, 3.8 mmol), and 4-(2-chloroethyl) morpholine hydrochloride (0.14 g, 0.7 mmol) in ethanol (10 ml) was refluxed for 8 h. The solvent was removed under reduced pressure, and the solid product was transferred in water, stirred, and filtered to yield **12** as white crystals.

Yield = 0.2 g (71 %); m.p. 122–125 °C; IR (cm^{-1}): 3015 (w, Ar–CH), 1625 (m, C = N); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.45 (t, 4H, J = 4.5 Hz, NCH_2) 2.76–2.81 (t, 2H, J = 6.8 Hz, $SCH_2\underline{CH_2}N$), 3.38-3.42 (t,, 2H, J = 6.8 Hz, $S\underline{CH_2}CH_2N$), 3.68-3.72 (t, 4H, J = 4.5 Hz, OCH_2) 7.37-7.99 (m, 5H, Ar–H), 8.28 (s, 1H, thiazole-H); MS(m/z): 375 [M^++1]. *Anal.* Calcd for: $C_{17}H_{18}N_4O_2S_2$: C, 54.53; H, 4.84; N, 14.96. Found: C, 54.55; H, 4.81; N, 14.86 .

Synthesis of 4-{2-[5-(2-phenylthiazole-4-yl)-1, 3, 4-oxadiazole-2-ylthio]ethyl}-dimethyl amine ***(13)***

A mixture of the oxadiazolinethione **11** (0.2 g, 0.7 mmol), fused sodium acetate (0.31 g, 3.8 mmol), and 2-dimethylaminoethyl chloride hydrochloride (0.11 g, 0.7 mmol) in ethanol (10 ml) was heated under reflux for 7 h. The solvent was removed under vacuum, and the solid was washed with water, filtered to yield compound **13** as white crystals.

Yield = 0.2 g (71 %); m.p. 111-114 °C; IR (cm^{-1}): 3030 (w, Ar–CH),1632 (m, C = N); ^{1}H NMR (DMSO-d_6,δ, ppm): 2.20 (s, 6H, $N(CH_3)_2$, 2.66–2.71 (t, 2H,

J = 7.0 Hz, $SCH_2\underline{CH_2}N$), 3.42–3.47 (t, 2H, J = 7.0 Hz, $S\underline{CH_2}CH_2N$), 7.47-8.04 (m, 5H, Ar–H), 8.06 (s, 1H, thiazole-H); MS(m/z): 333 [M^++1]. *Anal.* Calcd for: $C_{15}H_{16}N_4OS_2$: C, 54.19; H, 4.85; N, 16.85. Found: C, 54.12; H, 4.82; N, 16.86.

Synthesis of 5-(2-phenylthiazole-4-yl)-3-(piperidin-1-yl-methyl)-1, 3, 4-oxadiazole-2(3H)-thione ***(14)***

To a solution of 1, 3, 4-oxadiazol-5-thione **11** (0.2 g, 0.7 mmol) in ethanol, a mixture of formaldehyde (0.2 g, 0.14 mmol) and a piperidine (0.065 g, 0.7 mmol) in ethanol was added gradually with stirring. After complete addition, solution was stirred overnight at room temperature. The separated solid product was filtered, washed with water, dried, and crystallized from ethanol.

Yield = 0.15 g (60 %); m.p. 234–237 °C; IR (cm^{-1}): 3030 (w, Ar–CH), 1615 (m, C = N),1310 (m, C = S); ^{1}H NMR (DMSO-d_6,δ, ppm): 1.91–2.10 (m, 4H, piperidine-CH_2), 2.23–2.35 (m, 2H, piperidine-CH_2), 3.34-3.50 (t, 4H, J = 4.4 Hz, piperidine-CH_2), 4.89(s, 2H, N-CH_2-N), 7.45-8.10 (m, 5H, Ar–H), 8.27 (s, 1H, thiazole-H); MS(m/z): 359 [M^++1]. *Anal.* Calcd for: $C_{17}H_{18}N_4OS_2$: C, 56.96; H, 5.06; N, 15.63. Found: C, 56.89; H, 5.02; N, 15.60.

Synthesis of (5-amino-4-ethylester-1H-pyrazol-1-yl) (2-phenylthiazole-4-yl)-methanone ***(15)***

A mixture of **3** (0.25 g, 1.1 mmol) and ethyl(ethoxymethylene) cyanoacetate (0.19 g, 1.1 mmol) in ethanol (10 ml) was heated under reflux for 8 h. The reaction mixture was cooled, and solvent was removed under vacuum, and the solid residue was recrystallized from ethanol to yield compound **15** as white crystals.

Yield = 0.25 g (64 %); m. p. 166–169 °C; IR (cm^{-1}): 3340, 3190 (m, NH_2), 3030 (w, Ar–CH), 1730 (s, C = O), 1630 (m, C = N); ^{1}H NMR (DMSO-d_6,δ, ppm): 1.25-1.28 (t, 3H, J = 7.0 Hz, CH_3), 4.20–4.25 (q, 2H, J = 7.0 Hz, CH_2), 7.53-7.86 (m, 5H, Ar–H), 7.98 (bs, 2H, NH_2),8.04 (s, 1H, thiazole-H), 8.97 (s, 1H, pyrazole-H); MS(m/z): 343 [M^++1]. *Anal.* Calcd for: $C_{16}H_{14}N_4O_3S$: C, 56.13; H, 4.12; N, 16.36. Found: C, 56.12; H, 4.14; N, 16.26.

Synthesis of (5-amino-3-phenyl-1H-pyrazol-1-yl) (2-phenylthiazol-4-yl)-methanone ***(16)***

A mixture of **3** (0.25 g, 1.1 mmol) and benzoyl acetonitrile (0.145 g, 1.1 mmol) in absolute ethanol (10 ml) was refluxed for 5 h. After cooling, the solvent was removed by distillation, and the solid residue was recrystallized from ethanol to yield **16** as pale yellow crystals.

Yield = 0.30 g (77 %); m.p. 189-192 °C; IR (cm^{-1}): 3380, 3215 (m, NH_2), 3020 (w, Ar–CH), 1700 (s, C = O), 1610 (m, C = N); ^{1}H NMR (DMSO-d_6,δ, ppm): 7.25 (bs, 2H, NH_2), 7.40–7.56 (m, 5H, Ar–H), 7.83–7.89 (m, 5H, Ar–H), 8.04 (s, 1H, thiazole-H), 8.10 (s, 1H, pyrazole-H); MS(m/z): 347 [M^++1]; *Anal.* Calcd for $C_{19}H_{14}N_4OS$: C, 65.88; H, 4.07; N, 16.17. Found: C, 65.82; H, 4.10; N, 16.12.

Docking study

Focused compound-screening libraries are commonly used to improve the efficiency and productivity of early drug discovery efforts. Traditionally, each compound in a focused library is selected, based on structural and physical properties that will increase its probability of having activity for one specific target. In this study, we have extended this approach to identify potential multi-ligands as inhibitors. In order to find out the interactions of designed target molecules on COX-2 enzyme, the docking study was carried out on VLife MDS 4.0 software using grid analysis-based batch docking. The structures were drawn in Vlife engine ≫ tools ≫ draw 2D molecule and saved in dot mol format.

The 2D structures of the compounds were converted into the 3D structures using the software. The 3D structures were energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field. The appropriate COX-2 enzyme, i.e., 1CX2 was downloaded from www.resb.org. The enzyme was cleaned to remove the non-amino acid part using biopredicta software. In total, six cavities having hydrophobic surface area (Å) 13072.28, 13451.48, 8982.33, 2625.82, 2217.69, and 3251.47 were identified. The cavities in the receptor were mapped to assign an appropriate active site; the basic feature used to map the cavities was the surface mapping of the receptor and identifying the geometric voids as well as scaling the voids for its hydrophobic characteristics. All the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area. Cavity no. **1** having hydrophobic surface area 13072.28Å was selected for docking. The active site for docking was defined as all atoms within 5 Å radius. Using biopredicta ≫ tool ≫ docking ≫ grid docking, appropriate selections in the selected receptor and selected ligand panels were carried out. Docking was performed on selected cavity number 1 having hydrophobic surface area 13072.28 Å in the Grid over panel for the 1CX2 receptor. Batch docking results were recorded, and the images were saved to find out the proper conformations and binding interactions. The binding interactions were evaluated and identified by opening the receptor structure in MDS followed by the compound which was saved as ligand dock file. The molecule and receptor were merged together using merge option in the software. Then, the interactions were evaluated for this

complex, selected ligand and receptor structure using biopredicta tool. The standard COX-2 inhibitor indomethacine was used in study for comparison.

Biological activities

Synthesized compounds (**6–16**) were investigated for anti-inflammatory and analgesic activities and acute ulcerogenicity of the most active representatives (**9, 11, 14, 15**, and **16**) of the series. 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 six each.The test compounds and the standard drugs were administered in the form of a suspension (using1 % carboxymethylcellulose) in distilled 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 of 45–55 %, under a 12-h light–dark cycle, and they were fed standard animal feed. All the animals were allowed to acclimatize for a week before use.

Anti-inflammatory activity (Winter *et al.,* 1962)

Anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema method. A freshly prepared aqueous suspension of carrageenan (1.0 % w/v0.1 ml) was injected in the subplanter region of 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 were measured before injection of carrageenan for 0 min and measured again after 1, 2, and 3 h after carrageenan injection with the help of digital plethysmometer (UGO BASIL, ITALY). The edema was expressed as a mean reduction in paw volume (ml) after treatment with test compounds, and the percent of edema inhibition was obtained as follows:

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

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

Analgesic activity (Koster *et al.,* 1959)

Analgesic activity was evaluated using acetic acid-induced writhing method. After 50 min of the oral administration of test compound and standard drug, each animal was injected with 3 % (w/v) acetic acid solution intraperitoneally. After 10 min of acetic acid injection, the number of muscular contractions (writhing) in mice was counted for a period of 10 min. A significant reduction in the number of writhing by any treatment as compared to that of control animals was considered as a positive analgesic response. The average number of writhes in each group of the treated mice was compared to that of the control. The percentage analgesic activity was expressed according to the formula:

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

where n is the number of writhes in control group of mice, and n' is the number of writhes in test and standard groups of mice.

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 consisting of six animals. Control group of animals were administered only 1 % carboxymethylcellulose solution in water. One group was administered with diclofenac sodium at a dose of 25 mg/kg once daily for 4 days. The remaining group of animals were 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 the pylorus ligation procedure. Four hours after the ligation, the 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).

Statistical analysis

Data obtained for each set of anti-inflammatory model were expressed as mean of changes in paw volumes ± SEM and analyzed by one-way ANOVA followed by Dunnett's *t* test. Data from acetic acid-induced writhing test were expressed as mean of a number of writhes ± SEM and analyzed by one-way ANOVA followed by Dunnett's *t* test. All statistical calculations were performed using evaluation version of Graph Pad Prism 3.0 (USA) statistical software.

Conclusion

Synthesized thiazole derivatives exhibited significant analgesic and anti-inflammatory activities. Among all the synthesized compounds, those possessing 1, 3, 4-oxadiazole-2(3H)-thione (**11** and **14**) and pyrazole (**15** and **16**) at

position 4 of thiazole exhibited more prominent and consistent anti-inflammatory activity than that of the standard drug diclofenac sodium. The compound possessing 1, 3, 4-oxadiazole-2(3H)-one **(9)** showed moderate anti-inflammatory activity. These compounds (**9, 11, 14, 15**, and **16)** also showed significant analgesia in acetic acid-induced writhing test. The tested compounds showed one tenth of the ulcer index to that of reference diclofenac sodium at a dose of 25 mg/kg. The compounds 11 and 14 exhibited good activities both in silico(docking) and in vivo studies. Docking study and in vivo results showed that these series of compounds possess good potential and can be further developed into a potent lead.

Acknowledgments The authors are wholeheartedly grateful to Prof. T. J. Sawant, Founder Secretary, Jayawant Sikshan Prasarak Mandal's, Charak College of Pharmacy and Research, Wagholi, Pune for encouragements and support. Mr. Rishikesh Antre is being thanked here for his help in docking study. The authors are thankful to the Head, Department of Chemistry, Vinyakrao Patil Mahavidyala Vaijapur-423 701 (M.S.), India for partial support of this study. Government Medical College, Aurangabad for providing the experimental animals and Department of Analytical Chemistry, University of Pune; and SAIF, Chandigarh University, Chandigarh, India are thanked for providing spectra of compounds.

References

Amir M, Kumar H, Javed SA (2007) Non-carboxylic analogues of naproxen: design, synthesis, and pharmacological evaluation of some 1, 3, 4-oxadiazole/thiadiazole and 1, 2, 4-triazole derivatives. Arch der Pharmazie 340(11):577–585

Amit SK, Alan BM, Brenda CC et al (2000) Ester and amide derivatives of the nonsteroidal anti-inflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors. J Med Chem 43(15):2860–2870

Brown K, Cater DP, Cavalla JF, Green D, Newberry RA, Wilson AB (1974) Nonsteroidal anti-inflammatory agents. 1. 2, 4-diphenylthiazole-5-acetic acid and related compounds. J Med Chem 17(11):1177–1181

Clapp LB (1976) Adv Heterocycl Chem 20:65

Dannhardt G, Kiefer W (2001) Cyclooxygenase inhibitors: current status and future prospects. Eur J Med Chem 36:109–126

Diane HB, David TC et al (1993) 1,3,4-oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole analogs of the fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities. J Med Chem 36(13):1802–1810

Fu JY, Masferrer JL, Seibert K, Raz A, Needleman P (1990) The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. J Biol Chem 265:16737–16740

Ganguly AK, Bhatnagar OP (1973) Effect of bilateral adrenalectomy on production of restraint ulcers in the stomach of albino rats. Can J Physiol Pharmacol 51:748–750

Goyal RK, Chakrabarti A, Sanyal AK (1985) The effect of biological variables on the antiulcerogenic effect of vegetable plantain banana. Planta Med 29:85–88

Hall GE, James W (1966) Chemistry of micrococcin *P*. Part VIII. A method for the degradation of thiazole-4-carboxylic acids. J Chem Soc C: 1357–1360

Jouzeau JY, Terlain B, Abid A, Nedelec E (1997) Cyclooxygenase isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. Drugs 53:563–582

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

Leval X, Julémont F, Delarge J, Pirotte B (2002) New trends in dual 5-LOX/COX inhibition. Curr Med Chem 9:941–962

Manjunathaa K, Boja P, Prajwal LL, Jennifer F (2010) Synthesis and biological evaluation of some 1,3,4-oxadiazole derivatives. Eur J Med Chem 45:5225–5233

Palomer A, Cabre F, Espinosa A et al (2002) Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. J Med Chem 45(7):1402–1411

Sharma RN, Xavier FP et al (2009) Synthesis of 4-benzyl-1, 3-thiazole derivatives as potential anti-inflammatory agents: an analogue-based drug design approach. J Enzyme Inhib Med Chem 24(3):890–897

Shashikant VB, Kailash GB, Mayuresh KR, Ajit AP et al (2008) Design, synthesis and evaluation of antiinflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1,3,4-oxadiazole-2-thiol and schiff bases of diclofenac acid as nonulcerogenic derivatives. Bioorg Med Chem 16(4):1822–1831

Shay M, Komarov SA, Fels D, Meranze D, Grunstein H, Siplet H (1945) A simple method for the uniform production of gastric ulceration in the rats. Gastroenterol 5:43–61

Swain C (2007) N-Phenylamidines as 5-HT2A receptor antagonists. WO138343

Tally JJ, Bertenshaw RS, Brown DL, Seibert K et al (2000) N-[[(5-Methyl-3-phenylisoxazol-4-yl)-phenyl]sulfonyl]propanamide, sodium salt, parecoxib sodium: a potent and selective inhibitor of COX-2 for parenteral administration. J Med Chem 43(9):1661–1663

Winter CA, Risley EA, Nuss GN (1962) Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol 111:544–547