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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel pyrazolyl tetrazoles as selective COX-2 inhibitors

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Abstract A series of novel pyrazolyl tetrazoles were synthesized by introducing tetrazole moiety at the fourth position of 1,3-substituted pyrazole nucleus. Synthesis was carried out by cyclization of different pyrazolonitriles using sodium azide in the presence of triethylammonium chloride as phase transfer catalyst. The structures of the synthesized compounds were confirmed on the basis of physical and spectral data. Among the synthesized compounds, **4b** and **4e** displayed significant anti-inflammatory activity with no observable ulcerogenic effect when compared with diclofenac sodium. Furthermore, compounds **4b** and **4e** were found to have COX-2 selectivity with a ratio of 0.44 and 0.48, respectively.

Keywords Pyrazoles · Tetrazoles · Anti-inflammatory activity · Acute ulcerogenicity · In vitro COX inhibitor study

Introduction

Currently marketed non steroidal anti-inflammatory drugs (NSAIDs) have been associated with several side effects like gastrointestinal mucosal damage, bleeding, intolerance, renal toxicity, and hepatotoxicity. The pharmacological effects of NSAIDs occur due to inhibition of the membrane enzyme called cyclooxygenase (COX-1 and COX-2), which is involved in prostaglandin biosynthesis. COX-1 is predominantly expressed ubiquitously and constitutively, and it serves a housekeeping role in stomach and kidneys. By contrast, COX-2 is involved in the production of prostaglandin, mediating pain and supporting the inflammatory process. Gastric side effects associated with classical NSAIDs are attributed to the nonselective inhibition of the isoenzymes. Even the selective COX-2 inhibitors have been associated with the unexpected cardiovascular adverse effects. Thus, development of a reliable and selective anti-inflammatory agent with a fewer side effects is a major challenge to medicinal chemists.

Pyrazole template is considered as an important chemical entity of various physiological significances and pharmaceutical utility. Pyrazoles have drawn a greater attention due to their wide range of therapeutic activities including anticonvulsant (Abdel *et al.*, 2009), antidepressant (Abdel *et al.*, 2009), antitumor (Peng-cheng *et al.*, 2010), (Christodoulou *et al.*, 2010), (Lin *et al.*, 2007), antimicrobial (Bondock *et al.*, 2010), (Radi *et al.*, 2010), ACE inhibitor (Menichini *et al.*, 2010), antiviral (Osma *et al.*, 2009), anti-inflammatory (Barsoum and Girgis, 2009), (Adnan *et al.*, 2004) etc.

The well known selective COX-2 inhibitor, Celecoxib, and non steroidal antiphlogistic drug, Lonazolac, are good examples of pyrazoles containing anti-inflammatory drugs. According to literature, tetrazole and its derivatives are known to possess antibacterial (Adnan *et al.*, 2004), anti-inflammatory (Umarani *et al.*, 2010), antifungal (Ram Shankar *et al.*, 2004), cyclo-oxygenase inhibitor (Navidopour *et al.*, 2006), hypoglycemic (Gao *et al.*, 2010), and anticancer (Gundugola *et al.*, 2010) activities.

Our current strategy consists of designing a selective COX-2 inhibitor with a gastric safety profile which may allow the use of these new agents for long-term prophylactic use in certain chronic inflammatory diseases. Since tetrazole is bioisostere for –COOH group and is also metabolically more

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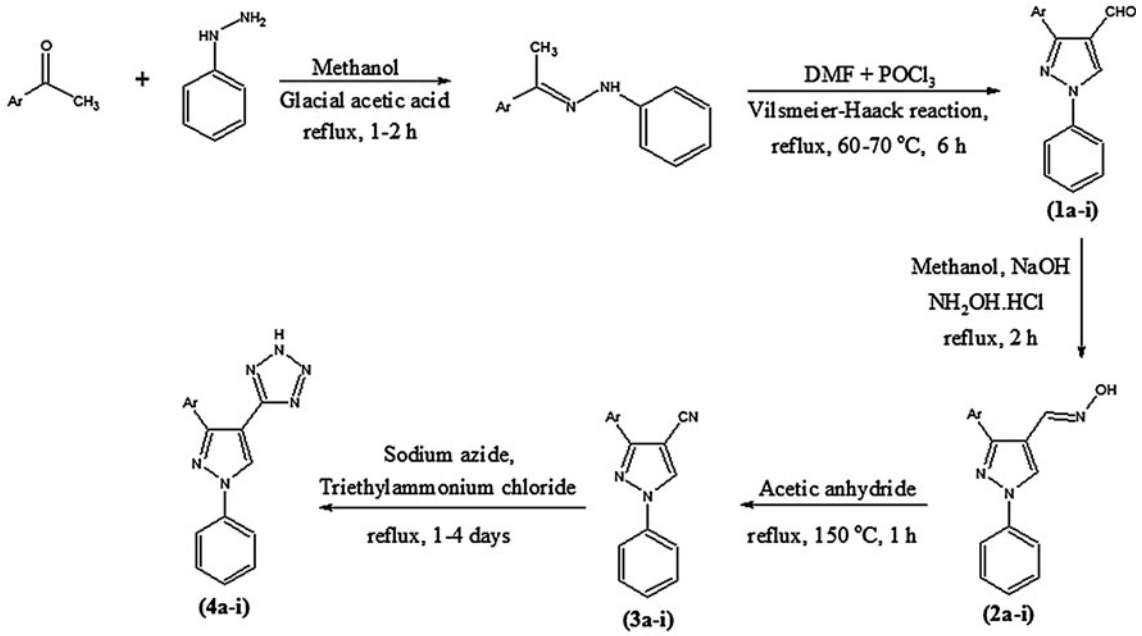
stable than the carboxylic acid group (Gupta *et al.*, 1999), in our present study, we made an attempt to synthesize title compounds as per Scheme 1 by replacing–CH_2COOH function in Lonazolac with tetrazole moiety in order to have better and more potent compounds which are not reported so far.

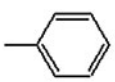
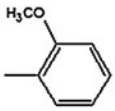
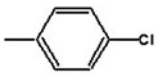
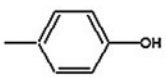
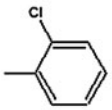
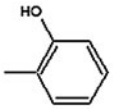
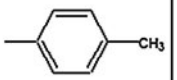
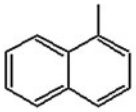
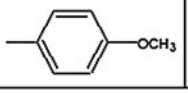
Experimental

Melting points (°C) were determined on Analab melting point apparatus by open capillary method and were uncorrected. The IR spectra were recorded on Shimadzu FTIR spectrometer using 1 % potassium bromide disks. ^{1}H NMR was recorded on Varian 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. ^{13}C NMR was recorded on Varian Gemini 400 MHz Spectrophotometer and mass spectra on Agilent 6430 triple quadruple LC–MS system. Thin layer chromatography was performed using E.Merck 0.25 mm silica gel plates, and visualization of spots was accomplished using UV light (256 nm).

General procedure for the synthesis of 1-phenyl-3-substituted phenyl-1H-pyrazole-4-carbaldehyde (**1a**–**i**)

A mixture of substituted acetophenone (0.01 mol) and phenyl hydrazine (0.01 mol) was taken in 15–20 ml of



Ar	Compound	Ar	Compound
a		f	
b		g	
c		h	
d		i	
e			

Scheme 1 General procedure for the synthesis of 5-(1-phenyl-3-substituted phenyl-1*H*-pyrazol-4-yl)-2*H*-tetrazoles (**4a**–**i**)

ethanol containing catalytic amounts of glacial acetic acid and heated at 50–60 °C for 1 h till a precipitate of phenyl hydrazone was obtained. The separated precipitate was filtered and recrystallized from ethanol. To an ice-cold solution of DMF (0.1 mol), phosphorus oxychloride (0.012 mol) was added drop-wise while maintaining the temperature below 10 °C. To this mixture, ice-cold solution of phenyl hydrazone (0.01 mol) in DMF was added lots wise, and stirring was continued under ice-cold condition for another half an hour. The reaction mixture was brought to room temperature and refluxed at 60–70 °C for 4–5 h. The reaction mixture was then cooled and poured into crushed ice with stirring and neutralized with aq. $NaHCO_3$ solution. The solid obtained was filtered and recrystallized from 90 % ethanol.

General procedure for the synthesis of 1-phenyl-3-substituted phenyl-1H-pyrazole-4-carbaldehyde oxime (**2a–i**)

A mixture of compound **1** (0.01 mol), hydroxylamine hydrochloride (0.02 mol), and sodium hydroxide (0.02 mol) was taken in ethanol and heated to reflux for 2 h. The reaction mixture was cooled, added to water, and neutralized with 0.1 N HCl. The separated solid was filtered and recrystallized from 90 % ethanol.

General procedure for the synthesis of 1-phenyl-3-substituted phenyl-1H-pyrazole-4-carbonitrile (**3a–i**)

Compound **2** (0.01 mol) and acetic anhydride (0.01 mol) were heated strongly for 1 h. The reaction mixture was cooled to room temperature and added to crushed ice with stirring. The separated solid was filtered and recrystallized from 60 % ethanol.

General procedure for the synthesis of 5-(1-phenyl-3-substituted phenyl-1H-pyrazol-4-yl)-2H-tetrazole (**4a–i**)

A mixture of carbonitrile **3** (0.01 mol), sodium azide (0.02 mol), triethylamine hydrochloride (0.02 mol) was taken in 15 ml of DMF and heated for reflux for 1–4 days by monitoring continuously with thin layer chromatography(TLC). The mixture was cooled and added to 50 ml of water and acidified with 0.1 N HCl. The separated solid was filtered and recrystallized from chloroform–ethanol mixture.

*5-(1,3-Diphenyl-1H-pyrazol-4-yl)-2H-tetrazole (**4a**)*

Light brown powder; m.p. 210–212 °C, yield, 60 %. IR [KBr] cm^{-1}: 3,412, 1,656, 1,623, 1,596; ^{1}H NMR (DMSO-d_6): δ 9.14(s, 1H, NH in pyrazole ring), 7.05–8.02 (m, 9H, Ar–H); ^{13}C NMR (DMSO-d_6):δ 119.26, 120.12, 128.21, 129.05, 130.12, 130.28, 131.20, 131.66, 134.76, 139.42, 150.08; Mass: *m/z* 288.6 [M^+]; Anal. Calcd. for $C_{16}H_{12}N_6$ (288.6): C, 66.56; H, 4.20; N, 29.15; Found: C, 66.04; H, 4.68; N, 29.25.

*5-[3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4b**)*

Light brown powder; m.p.230–232 °C, yield, 66 %. IR[KBr] cm^{-1}: 3,421, 1,653, 1,608, 1,595, 748;^{1}H NMR (DMSO-d_6): δ 9.12 (s, 1H, NH in pyrazole ring), 7.96 (d, 2H, $J = 7.85$ Hz, Ar–H), 7.79 (d, 2H, $J = 8.60$ Hz, Ar–H), 7.60 (t, 2H, $J = 7.82$ Hz, Ar–H), 7.30 (d, 2H, $J = 8.21$ Hz, Ar–H), 7.43(t, 1H, $J = 7.43$ Hz, Ar–H); ^{13}C NMR(DMSO-d_6):δ 119.45, 128.05, 129.11, 130.40, 130.43, 131.02, 131.50, 134.09, 139.33, 149.87; Mass: *m/z* 332.8 [M^+]; Anal. Calcd. for $C_{16}H_{11}ClN_6$ (332.8): C, 67.54; H, 4.67; N, 27.80. Found: C, 66.94; H, 4.68; N, 28.25.

*5-[3-(2-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4c**)*

Light brown powder; m.p.236–238 °C, yield, 62 %. IR[KBr] cm^{-1}: 3,424, 1,657, 1,602, 1,592, 738; ^{1}H NMR (DMSO-d_6): δ 9.16 (s, 1H, NH in pyrazole ring),7.92 (d, 2H, $J = 7.80$ Hz, Ar–H), 7.70 (d, 2H, $J = 8.64$ Hz, Ar–H), 7.63 (t, 2H, $J = 7.89$ Hz, Ar–H), 7.36 (d, 2H, $J = 8.20$ Hz, Ar–H), 7.42(t, 1H, $J = 7.42$ Hz, Ar–H);^{13}CNMR(DMSO-d_6):δ 119.30, 128.16, 129.12, 130.53, 130.66, 131.21, 131.62, 134.16, 139.12, 149.93; Mass: *m/z* 332.8 [M^+]; Anal. Calcd. for $C_{16}H_{11}ClN_6$ (332.8): C, 67.54; H, 4.67; N, 27.80. Found: C, 66.92; H, 4.64; N, 28.26.

*5-[3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4d**)*

Light brown powder; m.p. 220–222 °C, yield, 58 %. IR [KBr] cm^{-1}: 3,424, 2,932, 1,648, 1,596, 1,500; ^{1}H NMR (DMSO-d_6): δ 9.15 (s, 1H, NH in pyrazole ring), 8.13 (d, 2Ar–H, $J = 8.25$ Hz),7.80 (d, 2Ar–H, $J = 7.43$ Hz), 7.62 (m, 3Ar–H), 7.40 (m, 2Ar–H), 2.41 (s, 3H, CH_3);^{13}C NMR (DMSO-d_6):δ 21.83, 118.96, 119.62, 120.42, 129.45, 130.12, 130.65, 131.44, 131.76, 133.87, 134.06, 139.46, 150.45; Mass: *m/z* 302.3 [M^+]; Anal. Calcd. for $C_{17}H_{14}N_6$ (302.3): C, 67.54; H, 3.44; N, 26.04. Found: C, 67.94; H, 4.28; N, 26.85.

*5-[3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4e**)*

Yellow powder; m.p.240–242 °C, yield, 56 %. IR [KBr] cm^{-1}: 3,418, 1,651, 1,596, 1,500, 1,249, 1,180;^{1}H NMR (DMSO-d_6): δ 9.05(s, 1H, NH in pyrazole ring), 7.92 (d, 2Ar–H, $J = 7.85$ Hz), 7.70 (d, 2Ar–H, $J = 8.99$ Hz), 7.62 (t, 2Ar–H, $J = 8.09$ Hz), 7.41(t, 1Ar–H, $J = 7.43$ Hz), 7.05

(d, 2Ar–H, $J = 8.60$ Hz), 3.82(s, 3H, OCH_3); ^{13}C NMR(DMSO-d_6):δ 56.82, 119.12, 120.44, 120.68, 129.58, 130.42, 130.75, 131.76, 131.94, 134.92, 140.12, 148.62, 150.46 Mass: *m/z* 318.3 [M^+]; Anal. Calcd. for $C_{17}H_{14}N_6O$ (318.3): C, 64.14; H, 4.43; N, 26.40. Found: C, 64.04; H, 4.88; N, 26.95.

*5-[3-(2-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4f**)*

Yellow powder; m.p.244–246 °C, yield, 60 % IR [KBr] cm^{-1}: 3,422, 1,649, 1,593, 1,506, 1,238, 1,162;^1H NMR (DMSO-d_6): δ 9.18 (s, 1H, NH in pyrazole ring), 8.16 (d, 2Ar–H, $J = 7.82$ Hz), 7.84 (d, 2Ar–H, $J = 8.96$ Hz), 7.66 (t, 2Ar–H, $J = 8.12$ Hz), 7.39 (t, 1Ar–H, $J = 7.47$ Hz) , 7.03 (d, 2Ar–H, $J = 8.68$ Hz), 3.86(s, 3H, OCH_3); ^{13}C NMR(DMSO-d_6):δ 55.68,119.14,120.44,120.67, 129.68, 130.67, 130.86, 131.26, 131.98, 134.56, 139.66, 151.46 Mass: *m/z* 318.3 [M^+]; Anal. Calcd. for $C_{17}H_{14}N_6O$ (318.3): C, 64.14; H, 4.43; N, 26.40. Found: C, 64.10; H, 4.62; N, 26.66.

*4-[1-Phenyl-4-(2H-tetrazol-5-yl)-1H-pyrazol-3-yl] phenol (**4g**)*

Brown powder; m.p.250–252 °C, yield, 55 %.IR[KBr] cm^{-1}: 3,442, 3,316, 1,657, 1,596, 1,519; ^1H NMR (DMSO-d_6): δ 9.14 (s, 1H, NH in pyrazole ring), 7.98 (d, 2H, $J = 7.85$ Hz, Ar–H), 7.82 (d, 2H, $J = 8.60$ Hz, Ar–H), 7.76 (t, 2H, $J = 7.82$ Hz, Ar–H), 7.56 (d, 2H, $J = 8.21$ Hz, Ar–H), 7.43 (t, 1H, $J = 7.43$ Hz, Ar–H); ^{13}C NMR(DMSO-d_6):δ 119.23, 120.28, 120.88, 130.08, 130.66, 130.89, 131.22, 131.86, 134.12, 139.86, 146.12, 150.35; Mass: *m/z* 304.3 [M^+]. Anal. Calcd. for $C_{16}H_{12}N_6O$ (304.30): C, 63.15; H, 4.17; N, 24.84. Found: C, 63.74; H, 4.98; N, 23.65.

*2-[1-Phenyl-4-(2H-tetrazol-5-yl)-1H-pyrazol-3-yl] phenol (**4h**)*

Brown powder; m.p.254–256 °C, yield, 50 %.IR[KBr] cm^{-1}: 3,412, 3,386, 1,649, 1,598, 1,509; ^1H NMR(DMSO-d_6): δ 9.12 (s, 1H, NH in pyrazole ring), 7.89 (d, 2H, $J = 7.88$ Hz, Ar–H), 7.84 (d, 2H, $J = 8.65$ Hz, Ar–H), 7.72 (t, 2H, $J = 7.86$ Hz, Ar–H), 7.48 (d, 2H, $J = 8.28$ Hz, Ar–H), 7.44 (t, 1H, $J = 7.46$ Hz, Ar–H);^{13}C NMR(DMSO-d_6):δ 119.03,120.42, 120.58, 130.18, 130.86, 130.92, 131.42, 131.66, 134.32, 139.87, 148.22, 150.85; Mass: *m/z* 304.3 [M^+]. Anal. Calcd. for $C_{16}H_{12}N_6O$ (304.3): C, 63.15; H, 4.17; N, 24.84. Found: C, 63.46; H, 4.84; N, 23.64.

*5-[3-(2-Naphthyl)-1-phenyl-1H-pyrazol-4-yl]-2H-tetrazole (**4i**)*

Light brown powder; m.p. 250–252 °C, yield, 55 %. IR [KBr] cm^{-1}: 3,426, 1,648, 1,596, 1,500, 1,443; ^1H NMR(DMSO-d_6):δ 9.04(s,1H, NH in pyrazole ring), 7.02–8.06(m, 12H, Ar–H); ^{13}C NMR(DMSO-d_6): 119.08, 120.20, 120.44, 120.86, 128.44, 129.36, 130.45, 130.86, 131.60, 131.86, 134.66, 139.32, 150.86; Mass: *m/z* 338.3 [M^+]; Anal. Calcd. for $C_{20}H_{14}N_6$ (338.3): C, 70.99; H, 4.17; N, 24.84. Found: C, 71.24; H, 4.25; N, 24.65.

Biological activity

Anti-inflammatory activity

Adult albino rats of both sexes weighing between 120 and 150 g were used and divided into 11 groups of six animals each. The control group was given 1 % aq. CMC. Diclofenac sodium (20 mg/kg, standard) and test compounds (**4a–i**, 20 mg/kg) in 1 % aq. CMC were administered orally 1 h before induction of inflammation. Induction of inflammation was performed by s.c injection of 0.1 ml of freshly prepared 1 % carrageenan in saline solution, into the subplantar region of the right hind paw. The paw volume was measured using the plethysmometer at 0.5, 1, 2, 3 h after the carrageenan challenge. The average value of edema was calculated by taking the average of six animals at different hours. Percentage inhibition of edema was calculated for each group with respect to the control group.

Inflammation was expressed as the change in paw volume

$$\text{Edema} = T_t - T_0$$

where

T_0 Volume at '0' h
T_t Volume at 't' h

$$\text{Percentage reduction} = (V_0 - V_t)/V_0 \times 100$$

where,

V_0 Volume of the paw of control at time 't'
V_t Volume of the paw of drug treated at time 't'

Acute ulcerogenicity study

Young adult albino rats weighing 180–220 g were used for the experiment, and animals were divided into groups of six animals each. Rats were fasted 20 h before drug administration. The test compounds, diclofenac sodium and celecoxib, were given orally in doses of 100, 50, and 50 mg/kg, respectively, while the control group received only vehicle, i.e., 1 % CMC. Rats were fasted for 2 h, allowed to feed for 2 h, and then fasted for another 20 h. Rats were given another two doses in the second and third day. In the fourth day, rats were sacrificed, the stomach was removed, opened

along with the greater curvature, and rinsed with 0.9 % saline solution. The number of mucosal damage (red spots) was counted,and their severity (ulcerogenic severity) was graded from 0 to 4 according to the following score assignment. The following figures were calculated.

- % Incidence/10 = [no. of rats showing ulcer of any grade divided by total number of rats in the group × 100]/10.
- Average number of ulcers: number of ulcers in the group/total number of rats in the group.
- Average severity: Σ[each ulcer multiplied by its score of severity]/number of ulcers in the group.

Ulcer index = the sum of above three figures

	Score
Normal (no injury)	0
Latent small red spot	1
Wide red spot	2
Slight injury	3
Severe injury	4

In vitro COX study

The COX inhibition assay (Syed Nasir *et al.*, 2012) was performed according to the procedure mentioned in the 'Colorimetric COX (ovine) inhibitor Assay Kit' supplied by Cayman Chemical Company, MI, USA. While the reaction mixture of 100 % initial activity wells contained 150 µl of assay buffer, 10 µl of heme, and 10 µl of relative (COX-1or COX-2) enzyme solution, the reaction mixture of inhibitor wells was made of 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (COX-1 or COX-2), and 10 µl of the test samples. The plates were carefully shaken for few seconds and incubated for 5 min at 25 °C. After 5 min incubation, 20 µl of the colorimetric substrate solution was added to all the wells, followed by the addition of 20 µl of arachidonic acid to all the wells. The plates were shaken gently for few seconds and again incubated for 5 min at 25 °C. The absorbance of all the wells was read at 590 nm using Thermo make Automatic Ex-Microplate Reader. The COX inhibition activity (%) was calculated using the following formula:

$$\text{COX inhibition activity } (\%) = T/C \times 100$$

where T = absorbance of the inhibitor well at 590 nm, and C = absorbance of the 100 initial activity wells without inhibitor at 590 nm.

Results and discussion

Chemistry

The synthetic methodology followed to obtain the target compounds is outlined in Scheme 1. Cyclization of different phenyl hydrazones by Vilsmeier–Haack reaction afforded 1-phenyl-3-substituted phenyl-1H-pyrazole-4-carbaldehydes (**1a–i**), which on reaction with hydroxylamine hydrochloride gave pyrazole carbaldehyde oximes (**2a–i**). The heating of oximes with acetic anhydride at 140–150 °C gave pyrazolonitriles (**3a–i**). 1,3 Dipolar cyclization of compounds (**3a–i**) with sodium azide in the presence of triethylammonium chloride (acts as base and phase-transfer catalyst) gave pyrazolyl tetrazoles (**4a–i**). During the formation of pyrazolyl tetrazoles, there was variation in reaction time from 1 to 4 days depending on the substituent present in the phenyl ring. The conversion of pyrazolonitriles to tetrazoles was confirmed through IR spectra which showed disappearance of CN absorption band around 2,200–2,220 cm^{-1} and appearance of new band at around 3,400–3,440 cm^{-1} due to N–H. ^{1}H NMR spectrum of compound **4a** showed a singlet

Table 1 Anti-inflammatory activity of diclofenac sodium and synthesized compounds evaluated by carrageenan-induced paw edema

Compound	0.5 h	1 h	2 h	3 h	% Inhibition after 3 h
Control	2.5 ± 0.02	3.75 ± 0.01	4.25 ± 0.07	4.75 ± 0.01	
Diclofenac sodium	1.67 ± 0.04	1.15 ± 0.05	0.25 ± 0.03	0.54 ± 0.05	88
4a	1.1 ± 0.06	1.25 ± 0.07	1.45 ± 0.06	1.5 ± 0.01	68
4b	1.6 ± 0.06	1.2 ± 0.08	0.9 ± 0.02	0.70 ± 0.05	84
4c	0.9 ± 0.06	1.0 ± 0.08	1.15 ± 0.03	1.2 ± 0.02	75
4d	1.87 ± 0.06	2.21 ± 0.07	1.20 ± 0.06	1.50 ± 0.03	68
4e	1.78 ± 0.01	1.5 ± 0.06	1.1 ± 0.08	0.88 ± 0.05	81
4f	1.74 ± 0.03	1.35 ± 0.02	1.30 ± 0.06	1.6 ± 0.02	66
4g	0.75 ± 0.06	1.25 ± 0.06	1.3 ± 0.08	1.35 ± 0.05	72
4h	0.8 ± 0.01	1.15 ± 0.04	1.25 ± 0.05	1.30 ± 0.08	73
4i	1.78 ± 0.01	2.12 ± 0.04	1.84 ± 0.05	1.45 ± 0.08	70
	Edema (ml) (Mean ± ES)*				

Each value represents mean ± SE of six animals; statistical analysis was done by student's *t* test

* $P < 0.05$ when compared to control

at δ 9.14 due to pyrazole proton, and signals for aromatic protons appeared between δ 7.05 and 8.02, thus confirming the structure. Mass spectrum of compound **4a** displayed a molecular ion peak at m/z 288.6(M^+). The structure was further supported by ^{13}C NMR, and the signals appearing in the spectrum account for all the carbon atoms present in the molecule **4a**. Similarly, all other compounds were characterized, and the data were given in the experimental section.

Anti-inflammatory screening

In anti-inflammatory screening, among the nine tetrazole derivatives, compounds **4b** and **4e** were found to have good activity (above 80 % inhibition), which may be attributed to the presence of p-chloro and p-methoxy substitutions on phenyl ring at the third position of pyrazole nucleus, and the results are shown in Table 1.

Ulcerogenicity study

Compounds (**4b** and **4e**) which showed good anti-inflammatory activity in in vivo screening were further studied for gastric ulcerogenic potential in rats. Ulcerogenic effect was compared with classical NSAID, diclofenac sodium, and a COX-2 selective inhibitor, celecoxib. The results revealed that the test compounds were less ulcerogenic than diclofenac sodium and similar to celecoxib in ulcerogenic induction. Results are shown in Table 2.

COX-2/COX-1 selectivity assay

Compounds **4b** and **4e** were evaluated for their selectivity to inhibition of COX-2 and COX-1 isoenzymes using COX (ovine) inhibitor screening assay kit. The ratio of IC_{50} of COX-2 to IC_{50} of COX-1 (COX-2/COX-1) would suggest the selectivity of the compound and hence its gastric liability. The COX-2/COX-1 ratio of compounds **4b** and **4e** clearly indicated their selectivity toward COX-2 enzyme with a ratio of 0.44 and 0.48, respectively. The results are shown in Table 3.

Table 2 Ulcer index of synthesized compounds of the most active compounds, diclofenac sodium and celecoxib

Compound	% Incidence/10	Average no. of ulcer	Average severity	Ulcer index
Control	0	0	0	0
Diclofenac	10	5.60	1.42	17.02
Celecoxib	8	3.20	1.31	12.51
4b	8	4.30	1.70	13.90
4e	8	4.60	1.62	14.22

Table 3 COX-2/COX-1 ratio of compounds **4b**, **4e** and celecoxib

Compound	COX-2/COX-1
4b	0.44
4e	0.48
Celecoxib	0.02

Conclusion

In this investigation, nine new pyrazolyl tetrazoles (**4a–i**) were synthesized by isosteric replacement of $–CH_2COOH$ function in Lonazolac with tetrazole heterocycle, and the structures of resulting compounds were characterized on the basis of spectral data. All the compounds were screened for in vivo anti-inflammatory studies. Among the nine compounds, **4b** and **4e** exhibited most promising activity, and the activity was comparable with the standard drug, diclofenac sodium. Moreover, the same compounds **4b** and **4e** have less ulcerogenicity when compared with the diclofenac sodium. In COX-2/COX-1 selectivity assay, the same compounds exhibited more selectivity toward COX-2 enzyme.

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