Journal of Enzyme Inhibition and Medicinal Chemistry, 2011; 26(5): 742–748

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2010.548810

informa
healthcare

SHORT COMMUNICATION

Synthesis and biological evaluation of some new pyridazinone derivatives

Asif Husain[1], Sushma Drabu[1], Nitin Kumar[1], M. Mumtaz Alam[1], and Aftab Ahmad[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi, India and* [2]*Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India*

Abstract

A series of pyridazinone derivatives (**19–34**) were synthesized with an aim to synthesize safer anti-inflammatory agents. The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation (LPO) actions. The percentage inhibition in edema at different time intervals indicated that compounds **20**, **26**, **28** and **34** exhibited good anti-inflammatory potential, comparable with that of ibuprofen (85.77%) within a range of 67.48–77.23%. The results illustrate that 5-(4-fluoro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**26**) and 5-(4-chloro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**20**) showed best anti-inflammatory activity. Furthermore, activity is more in case of chloro substitution as compared with methyl-substitution. The compounds synthesized were also evaluated for their ulcerogenic and LPO action and showed superior gastrointestinal safety profile along with reduction in LPO as compared with that of the ibuprofen.

Keywords: Pyridazinone, anti-inflammatory, analgesic, lipid peroxidation

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world for the treatment of pain, fever and inflammation and disorders particularly arthritis[1,2]. Long-term use of the NSAIDs such as ibuprofen, indomethacin and naproxen exhibit gastric toxicity (gastrointestinal (GI) ulceration, bleeding) and nephrotoxicity[3]. The search for safer NSAIDs continues with the failure of anticipated 'Ideal' anti-inflammatory agents, the coxibs, on long-term usage[4,5]. Therefore, synthetic approaches based on chemical modification of NSAIDs have been taken with the aim of improving their safety profile.

Pyridazinones have been the subject of intensive synthetic investigations, because they possess a wide spectrum of pharmacological activities. Various 3-(2*H*)-pyridazinone derivatives have been described and their cardiotonic[6,7] anti-secretory and antiulcer[8] as well as analgesic and anti-inflammatory[9,10] activities have been investigated. 4-Ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone (emorfazone) and its derivatives have emerged as being of particular interest. Derivatives of N-substituted-4,6-diaryl-3-pyridazinone have emerged as potential analgesic anti-inflammatory and antipyretic agents[11].

As part of the continuing study for new anti-inflammatory and analgesics in our laboratories[12,13], a new series of pyridazinone derivatives were synthesized with an aim to obtain safer NSAIDs. The analgesic and anti-inflammatory activities were investigated for the title compounds by the acetic acid-induced writhing test and the carrageenan-induced rat paw edema test, respectively. Lipid peroxidation (LPO) and ulcerogenic actions of some of the selected compounds were also investigated and correlated.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC)

Address for Correspondence: Asif Husain, Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India. Tel.: +91-11-26059681, 26059688, ext. 5647, +91–989-1116086 (Mobile); Fax: +91-11-16988874. E-mail: drasifhusain@yahoo.com, ahusain@jamiahamdard.ac.in

(Received 05 August 2010; revised 13 December 2010; accepted 13 December 2010)

was carried out to monitor the reactions using silica gel (Merck No. 5554). The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1725X spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift (δ) values are reported in parts per million (ppm). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; m, multiplet. Mass spectroscopic analyses for compounds were performed on a JEOL JMS-D 300 instrument. Spectral data are consistent with the assigned structures. The molecular ion for compounds containing chloro-group was calculated according to ^{35}Cl isotope. Elemental analyses were performed on a Perkin-Elmer analyzer and were in range of ±0.4% for each element analyzed (C, H, N). Dry solvents were used throughout.

3-(4-Chloro/methyl benozyl)propionic acid (1,2)

The compounds, **1** and **2**, were synthesized according to the reported method[12].

3-Arylidene-5-(4-chloro/methyl phenyl)-2(3H)-furanones (3–18)

Compounds (**3**–**18**) were synthesized from 3-(4-chloro/methyl benozyl)propionic acid (**1,2**) following the literature method[12].

General procedure for the synthesis of 5-(substituted benzyl)-3-aryl-1,6-dihydro-6-pyridazinones (19–34)

2(3*H*)-Furanones (**3**–**18**) (0.005 mole) and hydrazine hydrate (1–2 mL) in *n*-propanol (5–6 mL) were refluxed for 3 h. After refluxing, reaction mixture was poured onto crushed ice, a precipitate was obtained, which was filtered, dried and recrystallized from methanol to give TLC pure 5-(substituted benzyl)-3-aryl-1,6-dihydro-6-pyridazinone derivatives.

5-Benzyl-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (19)

Yield: 59%, m.p. 174°C. ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 2H, CH_2), 7.16 (s, 1H, H-4, pyridazinone ring), 7.14–7.48 (m, 5H, benzyl ring), 7.42 and 7.73 (d each, 2xA_2B_2, *p*-substituted phenyl ring), 10.72 (s, 1H, NH). MS: *m/z* 296(M^+). IR (cm^{-1}, KBr): 3186 (NH), 2949 (CH), 1683 (CO), 718 (C–Cl). Anal calcd. for $C_{17}H_{13}ClN_2O$: C, 68.81; H, 4.42; N, 9.44. Found: C, 68.93; H, 4.45; N, 9.43.

5-(4-Chlorobenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (20)

Yield: 58%, m.p. 196°C. ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 2H, CH_2), 7.11 (s, 1H, H-4, pyridazinone ring), 7.23 (m, 4H, H-2,3,5,6, benzyl ring), 7.32 and 7.41 (d each, 2xA_2B_2, *p*-substituted phenyl), 10.97 (s, 1H, NH). MS: *m/z* 330(M^+). IR (cm^{-1}, KBr): 3179 (NH), 2942 (CH), 1688 (CO), 726 (CCl). Anal calcd. for $C_{17}H_{12}Cl_2N_2O$: C, 61.65; H, 3.65; N, 8.46. Found: C, 61.55; H, 3.67; N, 8.47.

5-(4-Nitrobenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (21)

Yield: 53%, m.p. 197°C. ^{1}H NMR ($CDCl_3$) δ 3.71 (s, 2H, CH_2), 6.78 (s, 1H, H-4, pyridazinone ring), 7.37 (m, 4H, H-2,3,5,6, benzyl ring), 7.53 and 7.62 (d each, 2xA_2B_2, *p*-substituted phenyl), 10.93 (s, 1H, NH). MS: *m/z* 341(M^+). IR (cm^{-1}, KBr): 3173 (NH), 2936 (CH), 1672 (CO), 707 (CCl). Anal calcd. for $C_{17}H_{12}ClN_3O_3$: C, 59.75; H, 3.54; N, 12.30. Found: C, 59.63; H, 3.57; N, 12.31.

5-(4-Hydroxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (22)

Yield: 57%, m.p. 192°C. ^{1}H NMR ($CDCl_3$) δ 3.59 (s, 2H, CH_2), 6.11 (m, 1H, OH), 7.06 (m, 2H, H-2,6, benzyl ring), 7.31 (s, 1H, H-4, pyridazinone ring), 7.47 and 7.71 (d each, 2xA_2B_2, *p*-substituted phenyl), 7.49 (m, 2H, H-3,5, benzyl ring), 9.41 (s, 1H, NH). MS: *m/z* 312(M^+). IR (cm^{-1}, KBr): 3178 (NH), 2942 (CH), 1686 (CO), 722 (C–Cl). Anal calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96. Found: C, 65.39; H, 4.17; N, 8.97.

5-(4-Methylbenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (23)

Yield: 59%, m.p. 186°C. ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 6.60 (s, 1H, H-4, pyridazinone ring), 7.13 and 7.36 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.34 (m, 2H, H-3,5, phenyl ring), 7.61 (m, 2H, H-2,4, phenyl ring), 10.93 (s, 1H, NH). MS: *m/z* 310(M^+). IR (cm^{-1}, KBr): 3173 (NH), 2939 (CH), 1684 (CO), 708 (C–Cl). Anal calcd. for $C_{18}H_{15}ClN_2O$: C, 69.57; H, 4.86; N, 9.01. Found: C, 69.53; H, 4.87; N, 9.03.

5-(4-Methoxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (24)

Yield: 61%, m.p. 169°C. ^{1}H NMR ($CDCl_3$) δ 3.42 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3), 6.81 (s, 1H, H-4, pyridazinone ring), 7.13 and 7.36 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.59 (m, 2H, H-3,5, phenyl ring), 7.68 (m, 2H, H-2,4, phenyl ring), 10.73 (s, 1H, NH). MS: *m/z* 326(M^+). IR (cm^{-1}, KBr): 3167 (NH), 3002 (CH), 1675 (CO), 717 (C–Cl). Anal calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57. Found: C, 66.23; H, 4.61; N, 8.55.

5-(4-Hydroxy-3-methoxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (25)

Yield: 53%, m.p. 191°C. ^{1}H NMR (DMSO) δ 3.48 (s, 2H, CH_2), 3.64 (s, 3H, OCH_3), 6.53 (s, 1H, H-4, pyridazinone ring), 7.07–7.29 (m, 3H, H-2,5,6, disubstituted benzyl ring), 7.46 and 7.71 (d each, 2xA_2B_2, *p*-substituted phenyl ring), 10.92 (s, 1H, NH). MS: *m/z* 342(M^+). IR (cm^{-1}, KBr): 3173 (NH), 2951 (CH), 1680 (CO), 713 (C–Cl). Anal calcd. for $C_{18}H_{15}ClN_2O_3$: C, 63.07; H, 4.41; N, 8.17. Found: C, 62.97; H, 4.39; N, 8.19.

5-(4-Fluorobenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (26)

Yield: 57%, m.p. 190°C. ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 2H, CH_2), 7.05 (s, 1H, H-4, pyridazinone ring), 7.10 and 7.26 (d each,

2xA_2B_2, *p*-substituted benzyl ring), 7.41 and 7.60 (d each, 2xA_2B_2, *p*-substituted phenyl ring), 11.14 (s, 1H, NH). MS: *m/z* 314(M+). IR (cm^{-1}, KBr): 3191 (NH), 2944 (CH), 1682 (CO), 719 (C–Cl). Anal calcd. for $C_{17}H_{12}ClFN_2O$: C, 64.87; H, 3.84; N, 8.90. Found: C, 64.93; H, 3.85; N, 8.91.

5-Benzyl-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (27)

Yield: 53%, m.p. 168°C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.59 (s, 2H, CH_2), 6.86 (s, 1H, H-4, pyridazinone ring), 7.02–7.43 (m, 5H, benzyl ring), 7.46 and 7.79 (d each, 2xA_2B_2, *p*-substituted phenyl ring), 8.92 (s, 1H, NH). MS: *m/z* 276(M^+). IR (cm^{-1}, KBr): 3185 (NH), 2952 (CH), 1676 (CO). Anal calcd. for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 10.14. Found: C, 78.23; H, 5.87; N, 10.17.

5-(4-Chlorobenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (28)

Yield: 48%, m.p. 188°C. ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.94 (s, 2H, CH_2), 7.24 (s, 1H, H-4, pyridazinone ring), 7.26 and 7.56 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.28–7.35 (m, 4H, H-2,3,5,6, phenyl ring), 10.69 (s, 1H, NH). MS: *m/z* 310(M^+). IR (cm^{-1}, KBr): 3174 (NH), 2939 (CH), 1683 (CO), 718 (C–Cl). Anal calcd. for $C_{18}H_{15}ClN_2O$: C, 69.57; H, 4.86; N, 9.01. Found: C, 69.53; H, 4.87; N, 8.99.

5-(4-Nitrobenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (29)

Yield: 47%, m.p. 189°C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.41 (s, 2H, CH_2), 7.13 (s, 1H, H-4, pyridazinone ring), 7.31 and 8.01 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.48 (m, 2H, H-3,5, phenyl ring), 7.81 (m, 2H, H-2,4, phenyl ring), 11.13 (s, 1H, NH). MS: *m/z* 321(M^+). IR (cm^{-1}, KBr): 3183 (NH), 2948 (CH), 1679 (CO). Anal calcd. for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.70; N, 13.08. Found: C, 67.23; H, 4.71; N, 13.07.

5-(4-Hydroxybenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (30)

Yield: 47%, m.p. 196°C. ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 3.43 (s, 2H, CH_2), 6.40 (s, 1H, H-4, pyridazinone ring), 6.63 (m, 2H, H-2,6, phenyl ring), 6.66 (m, 2H, H-2,6, benzyl ring), 6.79 (m, 2H, H-3,5, phenyl ring), 6.81 (m, 2H, H-3,5, benzyl ring), 12.25 (s, 1H, NH). MS: *m/z* 291(M^+). IR (cm^{-1}, KBr): 3173 (NH), 2957 (CH), 1685 (CO). Anal calcd. for $C_{18}H_{16}N_2O_2$: C, 73.96; H, 5.52; N, 9.58. Found: C, 73.98; H, 5.51; N, 9.57.

5-(4-Methylbenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (31)

Yield: 48%, m.p. 182°C. ^{1}H NMR ($CDCl_3$) δ 2.29 and 2.31 (s, each, 6H, 2xCH_3), 3.67 (s, 2H, CH_2), 6.51 (s, 1H, H-4, pyridazinone ring), 7.31 and 7.85 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.37 (m, 2H, H-3,5, phenyl ring), 7.59 (m, 2H, H-2,6, phenyl ring), 10.51 (s, 1H, NH). MS: *m/z* 289(M^+). IR (cm^{-1}, KBr): 3182 (NH), 2940 (CH), 1676 (CO). Anal calcd. for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.53; H, 6.27; N, 9.67.

5-(4-Methoxybenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (32)

Yield: 43%, m.p. 192°C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.67 (s, 3H, OCH_3), 3.97 (s, 2H, CH_2), 6.79 (s, 1H, H-4, pyridazinone ring), 7.37 and 7.82 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.49 (m, 2H, H-3,5, phenyl ring), 7.72 (m, 2H, H-2,6, phenyl ring), 11.15 (s, 1H, NH). MS: *m/z* 305(M^+). IR (cm^{-1}, KBr): 3186 (NH), 2944 (CH), 1682 (CO). Anal calcd. for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.53; H, 5.91; N, 9.13.

5-(4-Hydroxy-3-methoxy-benzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (33)

Yield: 51%, m.p. 180°C. ^{1}H NMR (DMSO) δ 2.39 (s, 3H, CH_3), 3.25 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2), 6.82 (s, 1H, H-4, pyridazinone ring), 7.04 (m, 1H, H-6, benzyl ring), 7.25 (m, 2H, H-3,5, phenyl ring), 7.53 (m, 1H, H-2, benzyl ring), 7.68 (m, 2H, H-2,6, phenyl ring), 7.75 (m, 1H, H-5, benzyl ring), 10.73 (s, 1H, NH). MS: *m/z* 321(M^+). IR (cm^{-1}, KBr): 3189 (NH), 2952 (CH), 1687 (CO). Anal calcd. for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.83; H, 5.65; N, 8.67.

5-(4-Fluorobenzyl)-3-(4-methylphenyl)-1,6-dihydro-6-pyridazinone (34)

Yield: 49%, m.p. 174°C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 7.01 (s, 1H, H-4, pyridazinone ring), 7.05 and 7.55 (d each, 2xA_2B_2, *p*-substituted benzyl ring), 7.21–7.29 (m, 4H, H-2,3,6,5, phenyl ring), 11.42 (s, 1H, NH). MS: *m/z* 293(M^+). IR (cm^{-1}, KBr): 3183 (NH), 2948(CH), 1672 (CO). Anal calcd. for $C_{18}H_{15}FN_2O$: C, 73.45; H, 5.14; N, 9.52. Found: C, 73.43; H, 5.17; N, 9.53.

Pharmacology

The protocol of the animal experiments was approved by the Institutional Animal Ethics Committee. The *in-vivo* anti-inflammatory activity of the synthesized compounds (**19**–**34**) was evaluated by carrageenan-induced rat paw edema method as described by Winter *et al.*[14]. Ibuprofen was taken as standard drug for comparison. All the compounds were administered orally and assayed at a dose of 20 mg/kg of body weight. The compounds that exhibited good anti-inflammatory activity (>65%) were further evaluated for their analgesic activity using acetic acid-induced writhing method[15]. The compounds that were screened for the analgesic activity were also tested for their ulcerogenic activity by the method reported by Cioli *et al.*[16] and LPO according to the method of Okhawa *et al.*[17]. The results of the pharmacological evaluation have been listed in Tables 1 and 2.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method of Winter *et al.*[14]. The experiment was performed on Albino rats of Wistar strain of either sex,

Table 1. Anti-inflammatory activity of the pyridazinone derivatives. 19–34.

Compound	R	R`	% Inhibition ± SEM[a] After 2 h	% Inhibition ± SEM[a] After 3 h
Control	—	—	—	—
Ibuprofen	—	—	75.23 ± 1.08	85.77 ± 0.75
19	4-Cl	H	23.09 ± 2.32***	36.99 ± 2.03***
20	4-Cl	4-Cl	54.52 ± 2.16***	71.95 ± 1.69***
21	4-Cl	4-NO_2	29.99 ± 2.95***	44.51 ± 1.80***
22	4-Cl	4-OH	27.62 ± 2.26***	41.05 ± 1.65***
23	4-Cl	4-CH_3	22.14 ± 3.08***	35.56 ± 2.54***
24	4-Cl	4-OCH_3	27.62 ± 2.35***	42.47 ± 1.73***
25	4-Cl	4-OH; 3-OCH_3	30.24 ± 2.84***	44.51 ± 2.17***
26	4-Cl	4-F	58.57 ± 1.04***	77.23 ± 0.68***
27	4-CH_3	H	20.71 ± 1.97***	36.58 ± 1.83***
28	4-CH_3	4-Cl	48.81 ± 2.25***	67.48 ± 1.68***
29	4-CH_3	4-NO_2	22.85 ± 2.33***	40.85 ± 2.61***
30	4-CH_3	4-OH	21.19 ± 1.93***	38.41 ± 2.63***
31	4-CH_3	4-CH_3	31.67 ± 1.70***	53.86 ± 1.73***
32	4-CH_3	4-OCH_3	38.09 ± 2.14***	60.57 ± 1.65***
33	4-CH_3	4-OH; 3-OCH_3	30 ± 2.50***	46.13 ± 1.76***
34	4-CH_3	4-F	46.19 ± 1.50***	68.90 ± 1.12***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[a]Relative to the standard (ibuprofen) and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.

Table 2. Analgesic activity along with ulcerogenic and lipid peroxidation effect of the pyridazinone derivatives (20, 26, 28 and 34).

Compound	R	R`	Analgesic activity: Number of writhings[b]	Analgesic activity: Inhibitory ratio[a] (%)	Lipid peroxidation[c]	Severity index[b]
Control	—	—	42.34 ± 1.45	—	0.239 ± 0.007[a]***	0.00 ± 0.00
Ibuprofen	—	—	16.34 ± 0.67***	61.03 ± 2.56	0.597 ± 0.007[ab]***	0.833 ± 0.105*
20	4-Cl	4-Cl	22.34 ± 0.67***	46.83 ± 2.78***	0.362 ± 0.008[ab]***	0.083 ± 0.083
26	4-Cl	4-F	21.5 ± 0.76***	48.97 ± 2.33***	0.342 ± 0.004[ab]***	0.083 ± 0.083
28	4-CH_3	4-Cl	24.34 ± 0.88***	42.12 ± 3.10***	0.318 ± 0.018[ab]***	0.25 ± 0.118
34	4-CH_3	4-F	23.5 ± 0.76***	44.22 ± 2.42***	0.418 ± 0.005[ab]***	0.25 ± 0.118

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
[a]Relative to the standard (ibuprofen) and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.
[b]Relative to their respective control and data were analyzed by one-way ANOVA followed by Turkey test for $n = 6$.
[c]Lipid peroxidation activity is expressed as nanomoles of MDA per milligram of protein.

weighing 180–200 g. The animals were randomly divided into two groups of six each. Group I was kept as control and received only 0.5% carboxymethyl cellulose (CMC) solution. Groups II was kept as standard and received ibuprofen (20 mg/kg, p.o.). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds (20 mg/kg) and standard drugs. The paw volume was measured by saline displacement shown on screen of digital Plethysmometer (Ugo Basile) at 2 and 3 h after carrageenan injection. Thus, the edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula:

Anti-inflammatory activity (% inhibition) $= \{(V_c - V_t)/V_c\} \times 100$

Analgesic activity

Compounds that showed good anti-inflammatory activity (>65%) were also screened for analgesic activity. Analgesic activity was done by acetic acid induce writhing method[15]. Swiss albino mice (25–30 g) of either sex were divided into different groups of six each. A 1% aqueous acetic acid solution (intraperitoneal injection in a volume of 0.1 mL) was used as writhing induced agent. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after oral administration of test drugs at a dose of 20 mg/kg. Group I was taken as control and received CMC suspension only, group II received reference drug ibuprofen and rest of the groups were treated with test drugs (20 mg/kg) suspended in 1.0% CMC orally. After 1 h of drug administration 0.10 mL of 1% acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of

the back, elongation of body and extension of hind limbs were counted for 5–15 min of acetic acid injection. The analgesic activity was expressed in terms of percentage inhibition.

$$\% \text{ Analgesic activity} = \{(n - n')/n\} \times 100$$

where n is mean number of writhes of control group and n' the mean number of writhes of test group.

Acute ulcerogenesis

Acute ulcerogenesis test was done according to the study of Cioli *et al.*[16] Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of test compounds or ibuprofen at the dose of 60 mg/kg. Control rats received oral administration of vehicle (suspension of 1% methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then killed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but <5, 3.0: ulcers > 5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

LPO in the gastric mucosa was determined according to the method of Ohkawa *et al.*[17]. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides and 10% of that tissue was homogenized at 10,000*g* in 1.8 mL of 1.15% ice-cold KCl solution. One millilitre of suspension medium was taken from the supernatant, 0.5 mL of 30% trichloroacetic acid followed by 0.5 mL of 0.8% thiobarbituric acid reagent were added to it. The tubes were covered with aluminium foil and kept in a shaking water bath for 30 min at 80°C. After 30 min, tubes were taken out and kept in ice cold water for 10 min. These were then centrifuged at 3000*g* for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against the blank on UV spectrophotometer.

The standard curve was used for estimating the concentration of malondialdehyde (MDA) prepared by using 1,1,3,3-tetraethoxypropane (Figure 1). The results are presented as nanomoles of MDA per milligram of protein (Table 2).

Result and discussion

Chemistry

2(3*H*)-Furanones (**3**–**18**) on reaction with hydrazine hydrate in *n*-propanol gave the title compounds, i.e. 5-(substituted-benzyl)-3-aryl-1,6-dihydro-6-pyridazinone derivatives (**19–34**). 2(3*H*)-Furanones (**3**–**18**) were prepared using 3-(4-substituted-benzoyl)propionic acid (**1**–**2**) following the previously reported methods of modified Perkin's reaction in higher yield[12]. The 3-(4-substituted-benzoyl)propionic acid (**1, 2**) was synthesized according to Friedal Craft's acylation reaction condition using substituted benzene (Scheme 1).

In the IR spectral data, all the compounds showed peaks each around 3180 (NH), 2940 (CH), 1685 (CO) cm^{-1}. The ^1H NMR spectra of the synthesized compounds showed characteristic peaks at appropriate δ values. The CH proton of fourth position of pyridazinone ring appeared as singlet around δ 6.60–7.26. The CH_2 proton appeared around δ 3.23–4.47 in aliphatic region. The NH proton appeared as a broad singlet appeared around δ 9.23–12.89. The structure of the compounds was further supported by mass spectral data. The synthesized compounds gave M+ peak in reasonable intensities. The molecular ion or other related ions produced the appropriate isotopic abundances due to the presence of chlorine atom(s).

Pharmacological evaluation

The pharmacological evaluation showed that the compounds are capable anti-inflammatory (Table 1) and analgesic agents with lesser GI toxicity and reduced LPO (Table 2).

The results illustrate that 5-(4-fluoro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**26**) and 5-(4-chloro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**20**) showed best anti-inflammatory activity, having maximum percentage inhibition in edema at different time intervals. Their percentage inhibition in edema at different time intervals, i.e. 77.23% (compound **26**) and 71.95% (compound **20**) was comparable with that of ibuprofen (85.77%). In addition, two more compounds, **28** and **34**, showed 67.48 and 68.90% inhibition, respectively. The remaining derivatives too possess

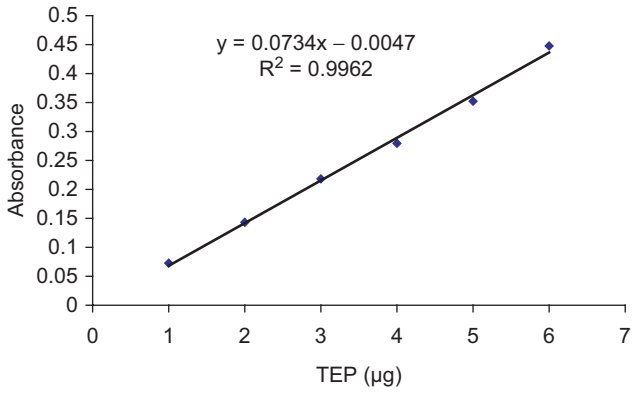
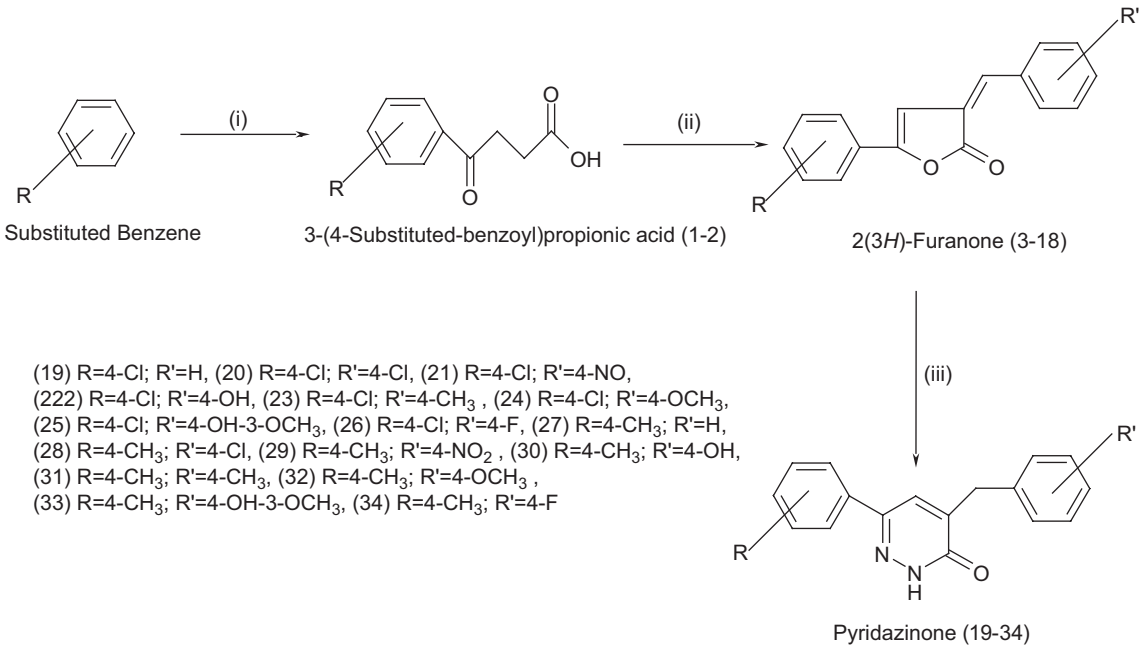


Figure 1. Calibration standard curve for TBARS concentration.



Scheme 1. Protocol for synthesis of title compounds.

anti-inflammatory activity but less than of the standard, ibuprofen.

The anti-inflammatory activity enhanced in the presence of electronegative substitution (chloro) and diminished in the presence of electropositive substitution (methyl) indicating that the activity declines with replacement of electronegative group to electropositive group.

Test compounds that exhibited good anti-inflammatory activity **20**, **26**, **28** and **34** were further evaluated for their analgesic, ulcerogenic and LPO actions. The results of analgesic activity indicated that compounds **20** and **26** showed 46.83% and 48.97% protection, respectively, against acetic acid-induced writhings and this percentage protection was comparable with that of ibuprofen (61.03%). Compounds **28** and **34** also showed good analgesic activity with 42.12% and 44.22% protection, respectively. According to structure–activity relationship (SAR), it is clear that 5-(4-fluoro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**26**) and 5-(4-chloro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**20**) were found to be a good anti-inflammatory agents with significant analgesic activity.

The tested compounds showed low ulcerogenic activity ranging from 0.1 ± 0.1 to 0.2 ± 0.12, whereas the standard drug ibuprofen showed high severity index of 0.8 ± 0.1. The maximum reduction in ulcerogenic activity (0.1 ± 0.1) was found in the compounds **26** and **20**. The LPO was measured as nanomoles of MDA per milligram of protein. Ibuprofen exhibited high LPO 0.597 ± 0.007, whereas control group showed 0.239 ± 0.007.

Thus, these compounds showed superior GI safety profile along with reduction in LPO in comparison with ibuprofen. The other tested compounds also exhibited better GI safety profile as compared with the standard drug ibuprofen.

In summary, 5-(4-fluoro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**26**) and 5-(4-chloro-benzyl)-3-(4-chloro-phenyl)-1,6-dihydro-6-pyridazinone (**20**) were found to have dual functional, i.e. anti-inflammatory and analgesic properties and hence, can be a promising class of compounds with an interesting pharmacological profile. It is conceivable that these derivatives could be further modified to develop potent and safer anti-inflammatory and analgesic agents. Further studies to acquire more information about quantitative SAR are in progress in our laboratory.

Acknowledgements

The authors thank UGC for financial assistance. Thanks are also due to In-charge animal house, Jamia Hamdard, New Delhi, for providing animals for pharmacological studies.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Sorbera LA, Leeson P, Castaner J, Castaner RM. Valdecoxib and parecoxib sodium: analgesic, antiarthritic, cyclooxygenase-2 inhibitor. Drugs Future 2001;26:133–140.
2. Palomer A, Cabré F, Pascual J, Campos J, Trujillo MA, Entrena A et al. Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. J Med Chem 2002;45:1402–1411.
3. Motawi TK, Abd Elgawad HM, Shahin NN. Modulation of indomethacin-induced gastric injury by spermine and taurine in rats. J Biochem Mol Toxicol 2007;21:280–288.
4. Verrico MM, Weber RJ, McKaveney TP, Ansani NT, Towers AL. Adverse Drug Events Involving COX-2 Inhibitors. Ann Pharmacother 2003;37:1203–1213.
5. Dogné JM, Supuran CT, Pratico D. Adverse cardiovascular effects of the coxibs. J Med Chem 2005;48:2251–2257.
6. Okushima H, Narimatsu A, Kobayashi M, Furuya R, Tsuda K, Kitada Y. A novel class of cardiotonics. Synthesis and pharmacological properties of [4-(substituted-amino)phenyl]pyridazinones and related derivatives. J Med Chem 1987;30:1157–1161.
7. Robertson DW, Krushinski JH, Beedle EE, Wyss V, Pollock GD, Wilson H et al. Dihydropyridazinone cardiotonics: the discovery and inotropic activity of 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2H -indol-2- one. J Med Chem 1986;29:1832–1840.
8. Yamada T, Nobuhara Y, Shimamura H, Yoshihara K, Yamaguchi A, Ohki M. Studies on new antiulcer agents. I. Synthesis and antisecretory activity of pyridazine derivatives. Chem Pharm Bull 1981;29:3433–3439.
9. Matsuo T, Tsukamoto Y, Takagi T, Sato M. Synthesis and biological activity of pyridazinooxazines. Chem Pharm Bull 1982;30:832–842.
10. Rubat C, Coudert P, Couquelet J, Bastide P, Bastide J. Synthesis and analgesic effect of N-substituted 5-arylidene-6-methyl-3-(4H)-pyridazinones. Chem Pharm Bull 1988;36:1558–1561.
11. Rubat C, Coudert P, Tronche P, Bastide J, Bastide P, Privat AM. Synthesis and pharmacological evaluation of N-substituted 4,6-diaryl-3-pyridazinones as analgesic, antiinflammatory and antipyretic agents. Chem Pharm Bull 1989;37:2832–2835.
12. Husain A, Alam MM, Siddiqui N. Synthesis, reactions and biological activity of 3-arylidene-5-(4-methylphenyl)-2(3H)-furanones. J Serb Chem Soc 2009;74:103–115.
13. Alam MM, Husain A, Hasan SM, Suruchi, Anwer T. Synthesis and pharmacological evaluation of 2(3H)-furanones and 2(3H)-pyrrolones, combining analgesic and anti-inflammatory properties with reduced gastrointestinal toxicity and lipid peroxidation. Eur J Med Chem 2009;44:2636–2642.
14. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 1962;111:544–547.
15. Siegmund E, Cadmus R, Lu G. A method for evaluating both non-narcotic and narcotic analgesics. Proc Soc Exp Biol Med 1957;95:729–731.
16. Cioli V, Putzolu S, Rossi V, Scorza Barcellona P, Corradino C. The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. Toxicol Appl Pharmacol 1979;50:283–289.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.