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Short communication

3-*O*-Substituted benzyl pyridazinone derivatives as COX inhibitors☆

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Abstract

New 3-*O*-substituted benzyl pyridazinone compounds have been synthesised and evaluated for their cyclooxygenase inhibitory activity and COX-2 selectivity. Among the compounds synthesised, three compounds (**11b**–**11d**) have shown in vitro COX-2 selectivity. These compounds have been evaluated for their in vivo potential using carrageenan-induced rat paw edema assay. One compound (**11b**) showed 32% anti-inflammatory activity at 30 mg kg^{-1} dose.

Keywords: 3-*O*-Substituted benzyl pyridazinone; COX inhibitors; Anti-inflammatory activity

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, inflammation, rheumatoid arthritis and osteoarthritis. The common dose limiting toxicity of NSAIDs is the increased risk of gastrointestinal ulceration, perforation and haemorrhage [1]. The enzyme cyclooxygenase (COX) catalyses the biooxygenation of arachidonic acid to prostaglandin G_2, which serves as a precursor for the synthesis of prostaglandins, prostacyclins and thromboxanes which are collectively termed as prostanoids [2]. The cyclooxygenase activity of the enzyme is the site of action of NSAIDs [3,4]. However, inhibition of prostanoid biosynthesis is associated with side effects such as ulceration and impairment of renal functions [5]. It has been well established that the cells express two isoforms of cyclooxygenases, namely cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [6]. COX-1 is expressed in many normal tissues and is the major form present in platelets, kidneys, gastrointestinal tract and play a key role in physiological processes [7], whereas COX-2 is an inducible form by many pro-inflammatory cytokines and mitogens. The second isoform is generally not detectable in normal tissues, but is elevated in inflammatory condition [8] and is also implicated in colon cancers [9], and Alzheimer's disease [10]. COX-2 is also constitutively expressed in kidneys [11], brain [12] spinal cord [13] and in mucosa of stomach [14]. As most of the NSAIDs inhibit both the isoforms of COX enzyme, currently the major focus of inflammatory research is to discover agents, which selectively inhibit the inducible COX-2 with little or no effect on COX-1. Synthesis of selective COX-2 inhibitors has been made possible due to X-ray crystal structure of COX enzyme [15]. Recently, Allen et al. [16] [17] claimed 3-aroyl benzyl pyridazinones with the following general formula as selective COX-2 inhibitors. The compounds, even though have shown good COX-2 activity in in vitro, the in vivo activity was not comparable to that of selective COX-2 inhibitors such as celecoxib and rofecoxib. In the present

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communication we describe our exploratory study to modify the structural motif of this compound by incorporating hetero atoms like N and O with an assumption that these may provide additional sites of interaction, thus giving the desired selectivity with increased potency Fig. 1. The compounds synthesised were initially tested for their in vitro COX inhibition at 100 μM followed by determination of IC_{50} for COX-1/COX-2 activity for interesting compounds.

2. Chemistry

Synthesis of these compounds is very concise and is outlined in Fig. 2. In the first step commercially available 3-methoxy benzaldehyde (**1**), was reduced with sodium borohydride to yield 3-methoxy benzyl alcohol (**2**), which was converted to the chloride **3** with thionyl chloride in dichloromethane and further to the nitrile **4** using potassium cyanide in dimethyl sulphoxide at room temperature. The obtained 3-methoxy benzo nitrile (**4**) was condensed with 3,6 dichloropyridazine to yield 2-(6-chloro-3-pyridazinyl)-2-(3-methoxyphenyl) acetonitrile (**5**) which on hydrolysis and decarboxylation gave 3-(3-methoxy benzyl)-1,6-dihydro-6-pyridazinone (**6**) which was demethylated using 48% aq. HBr. After purification by chromatography on silica gel 3-(3-hydroxybenzyl)-1,6-dihydro-6-pyridazinone (**7**) was isolated. Now this intermediate was reacted with various phenacyl bromides **8a**–**j** in the presence of sodium hydride in dimethyl formamide to obtain compounds **9a**–**j**. On the other hand, compounds **11a**–**e** were synthesised by reacting **7** with various *N*1-phenyl-2-chloro acetamides **10a**–**e** (prepared from substituted anilines and chloroacetyl chloride in presence of triethyl amine) in the presence of sodium hydride in DMF.

3. Results and discussion

The compounds synthesised were screened at 100 μM using in vitro spectrophotometric peroxidase assay in the initial screens for potency and COX-2 selectivity. Subsequently selected compounds exhibiting COX-2 selectivity were further evaluated and IC_{50} values were determined for COX-1 and COX-2. The COX-1 activity was assessed using Ram seminal vesicles as a source for COX-1 enzyme, whereas for COX-2 the enzyme source was the human recombinant cyclooxygenase-2 expressed in Sf9 cells infected with baculo virus. The test compounds were dissolved in DMSO and the enzyme activity was measured by the estimation of *N*,*N*,*N*,*N*-tetramethyl-*p*-phenylene diamine (TMPD) oxidation during the reduction of PGG_2 to PGH_2. Compounds, which showed better in vitro activity, were tested for their in vivo activity in carrageenan-induced rat paw edema model.

Using I and II as templates, a limited study has been initiated by changing substituents on phenyl ring of the phenacyl moiety in template I where X is oxygen. All these compounds were tested in vitro. In general, introduction of a bromo or fluoro in the fourth position of phenacyl ring resulted in desired COX-2 inhibition. This inhibitory activity observed was more in the case of bromo substituent **9a**. When the substituent was fluoro (**9e**) COX-2 specificity was observed to some extent. Other substituents like methyl (**9c**), *tert*-butyl (**9d**), have shown poor COX-2 selectivity at 100 μM concentration. In contrast, disubstituted compounds like 4-chloro-3-methyl (**9g**), 4-fluoro-3-methoxy (**9h**), and 2,4-dichloro (**9i**) analogues failed to show appreciable COX activity.

At this point further modification was carried out to synthesise A ring-substituted analogues of template II.

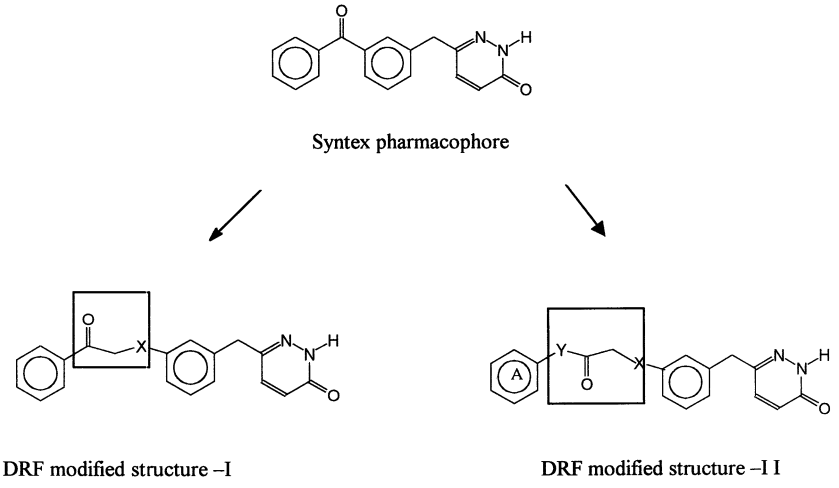


Fig. 1.

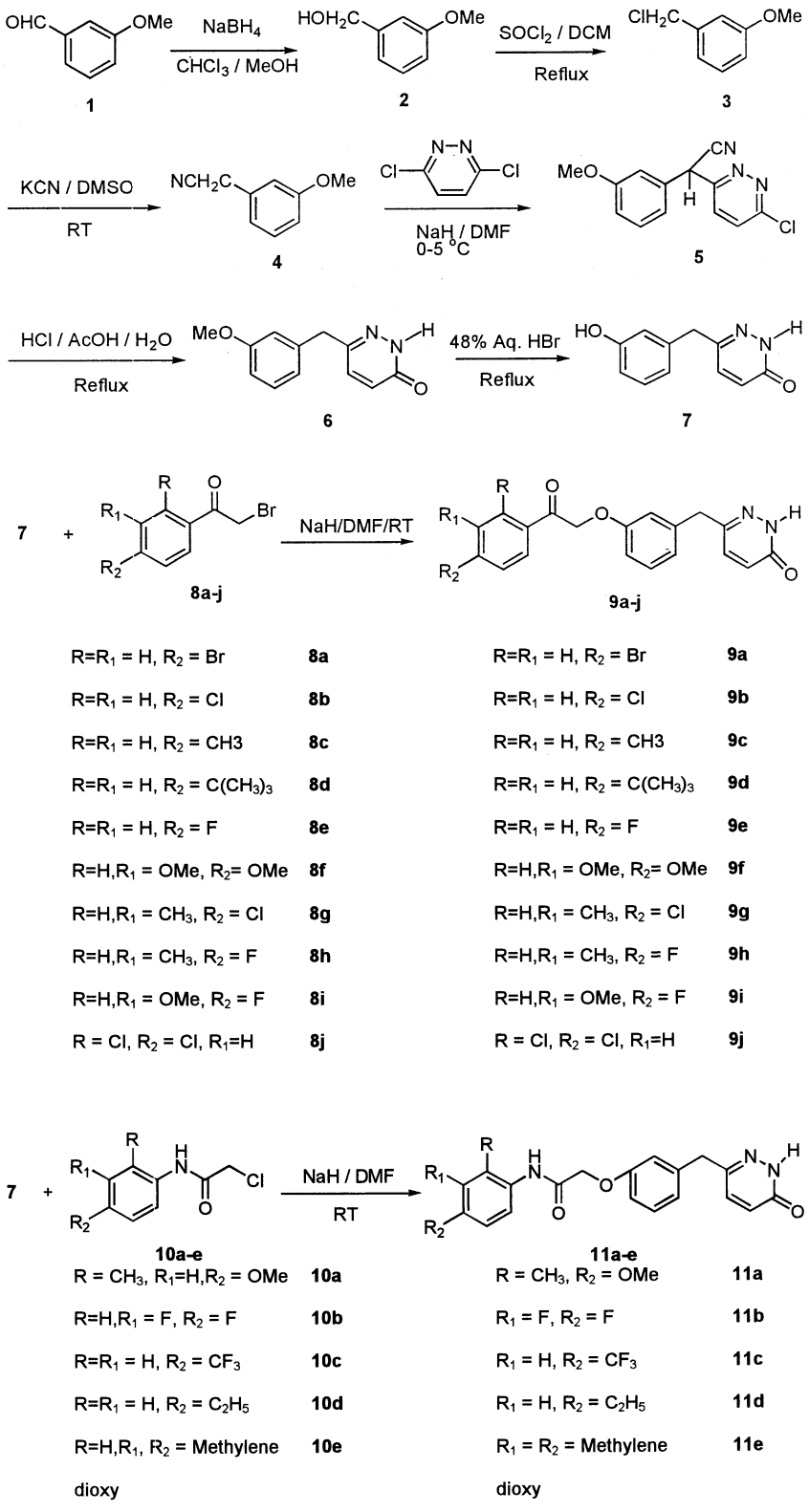
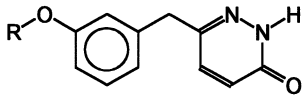


Fig. 2. Synthetic routes to compounds **9a**–**j** and **11a**–**e**.

Even though no clear SAR has been evolved, four compounds **11a**–**d** have shown better COX-2 selectivity at 100 μM concentration. These compounds were further tested and IC_{50} values were determined for COX-1 and COX-2. These results are summarised in Table 1. Three of these compounds (**11b**–**11d**), which showed desired COX-2 inhibition were evaluated further in carrageenan-induced rat paw edema model at 30

Table 1
COX-1 and COX-2 inhibitory data at 100 μM concentration



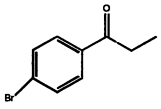
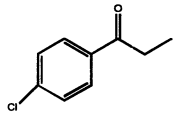
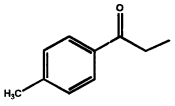
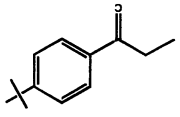
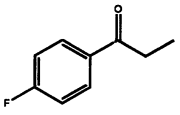
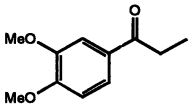
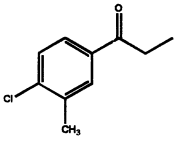
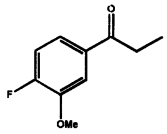
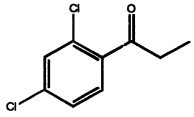
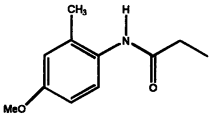
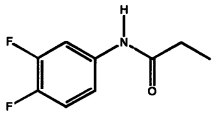
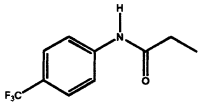
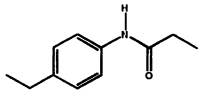
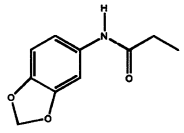
Compound	R	% Inhibition at 100μM*		IC_{50} values(μM)**		Ratio of IC_{50}
		[a]COX-1	[b]COX-2	COX-1	COX-2	COX-2/COX-1
9a		91	98	45	7.8	0.173
9b		31	0	NT	NT	
9c		41	0	NT	NT	
9d		46	48	NT	NT	
9e		47	100	>100	53	0.53
9f		39	28	NT	NT	
9g		49	0	NT	NT	
9h		0	0	NT	NT	

Table 1 (*Continued*)

9i		0	0	NT	NT	
11a		40	100	>100	10	0.1
11b		97	100	52	0.45	0.0086
11c		100	100	57	0.43	0.0075
11d		49	100	100	0.31	0.0031
11e		6	12	--	--	
Celecoxib		--	--	10.7	0.036	0.00336
Rofecoxib***		--	--	>500	0.321	0.00064
Indomethacin				0.067	7.8	116.4

NT = Not tested.
Enzyme source: [a] COX-1-Ram seminal vesicles; [b] COX-2-Human (expressed in Sf9 cells using baculo virus).
*Average of three determinations at 100 μM.
**IC_{50} tested at seven concentrations namely 0.1, 0.3, 1.0, 3.0, 10, 30 and 100 μM.
***Rofecoxib was tested up to 500 μM for COX-1 activity.

mg kg^{-1} by oral route (Table 2). Only one compound **11b** showed 32% inhibition at this dose whereas other derivatives in spite of having good in vitro COX-2 activity were found inactive in in vivo screen.

In conclusion novel 3-*O*-substituted benzyl pyridazinone derivatives were synthesised and screened for their in vitro COX-2 selectivity and in vivo anti-inflammatory activity. Even though few compounds have shown good in vitro COX-2 selectivity, this was not translated in in vivo studies.

4. Experimental protocols

4.1. Chemistry

The reported molecules were synthesised in the drug discovery Department of Dr. Reddy's research foundation. All other chemicals and reagents used in the synthesis were of reagent grade. Melting points were determined in an Electro thermal melting point apparatus (Buchi 535) and were uncorrected. Pre-coated silica gel plates (20 × 20 cm, silica gel 60 F_{254}, Merck) were used for TLC. ^{1}H-NMR spectra were recorded in a Varian-200 MHz, Gemini-200-software spectrometer (Varian USA). TMS was used as an internal standard and chemical shifts are given in ppm. IR spectra were recorded in a Perkin–Elmer 1600 series FT-IR (Perkin–Elmer, USA). Mass spectra were recorded in a Hewlett Packard 5989-A mass spectrometer. Starting materials were either commercially available or synthesised according to known literature methods.

4.1.1. General procedure for the synthesis of the intermediates **9a–j**

Bromine (1 mol) was added at room temperature to the appropriate acetophenone (1 mol) in 10 mL of acetic acid. Subsequently 0.5 mL of 48% aq. HBr was added and the contents were stirred at room temperature (r.t.) for 4 h. The reaction mixture was poured into ice and the precipitated solid was filtered, washed with water and dried under vacuum to get the phenacyl bromide.

4.1.2. General procedure for the synthesis of intermediates **10a–e**

Triethyl amine (1 mol) and chloroacetyl chloride (1 mol) were added at 0–5 °C to the appropriately substituted aniline (1 mol) in dichloromethane. The contents were stirred at 5 °C for 0.5 h and the precipitated solid was filtered and washed with diethyl ether to yield *N*1-phenyl-2-chloro acetamides as solid.

4.1.3. Synthesis of 2-(6-chloro-3-pyridazinyl)-2-(3-methoxyphenyl) acetonitrile (**5**)

To 3-methoxy benzonitrile (**4**) (5.0 g) in dry DMF (25 mL), at 0–5 °C was added 60% sodium hydride (1.14 g, 0.0341 mol). The contents were stirred there for 5 min and 3,6-dichloro pyridazine (5.025 g, 0.0341 mol) was added at one lot. The reaction mixture was stirred at 5–10 °C for 2 h, poured into ice and extracted with dichloromethane. The organic layer was washed with water, dried and concentrated. The residue was purified by chromatography over 100–200 mesh silica gel. Elution with 30% ethyl acetate in petroleum ether yielded the pure compound as a colourless solid (2.6 g, 30%).

4.1.4. Synthesis of 3-(3-methoxybenzyl)-1,6-dihydro-6-pyridazinone (**6**)

2-(6-Chloro-3-pyridazinyl)-2-(3-methoxyphenyl) acetonitrile (**5**) (2.5 g, 0.009 mol) was suspended in a 20 mL mixture of 2:1:1 HCl, acetic acid and water. The contents were refluxed for 12 h, cooled to r.t. and extracted with dichloromethane. The organic layer was washed with water and concentrated. The residue was filtered and washed with diethyl ether to get the pyridazinone as a colourless solid (1.35 g, 67.5%).

4.1.5. Synthesis of 3-(3-hydroxybenzyl)-1,6-dihydro-6-pyridazinone (**7**)

3-(3-Methoxybenzyl)-1,6-dihydro-6-pyridazinone (3.0 g, 0.0138 mol) was taken in 48% aq. HBr (15 mL) and the contents were refluxed for 2 h. The reaction mass was poured into ice and extracted with ethyl acetate. The ethyl acetate extract was washed with water and concentrated. The residue was purified by chromatography over 100–200 mesh silica gel. Elution of the column with 80% ethyl acetate in petroleum ether resulted in the pure compound as a pale brown solid (1.7 g, 60.9%)

4.1.6. Synthesis of 1-(4-bromophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9a**)

3-(3-Hydroxybenzyl)-1,6-dihydro-6-pyridazinone (0.310 g, 0.00154 mol) was dissolved in dry DMF (5 mL), 60% NaH (0.160 g, 0.00308 mol) was added at 5 °C, then 4-bromophenacyl bromide (0.427 g, 0.00154 mol) was added and contents were stirred at r.t. for 15 min. The reaction mixture was poured on ice, extracted with dichloromethane, washed with water, the organic layer was dried over sodium sulphate and concentrated to dryness. The residue was purified by chromatography over 100–200 mesh silica gel. Elution with 75% ethyl acetate in petroleum ether afforded the pure compound. Yield (32.4%); m.p. 218–219 °C; IR (KBr, cm^{-1}): 3262, 2928, 1696, 1661, 1580, 1524, 1484, 1460, 1349, 1281, 1223, 1153, 1107, 1072; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ_H: 8.8 (1H, s, –NH), 7.9 (2H, d, $J = 6.6$ Hz, phenylethanone), 7.7 (2H, d, $J = 6.6$ Hz, phenylethanone), 7.2 (1H, d, $J = 9.4$ Hz, pyridazine), 7.1 (1H, m, phenoxy), 6.8 (1H, d, $J = 9.4$ Hz, pyridazine), 6.6 (3H, m, phenoxy), 5.5 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$). MS; m/z (DIP): 400 [M^+], 291, 215, 185 (100%).

4.1.7. Synthesis of 1-(4-chlorophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9b**)

Yield (22.7%); m.p. 222–223 °C; IR (KBr, cm^{-1}): 3276, 2939, 1696, 1661, 1580, 1525, 1486, 1460, 1352, 1281, 1225, 1153, 1096, 1006; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ_H: 8.6 (1H, s, –NH), 7.9 (2H, d, $J = 8.4$ Hz, phenylethanone), 7.5 (2H, d, $J = 8$ Hz, phenylethanone), 7.3 (1H, m, phenoxy), 7.2 (1H, d, $J = 9.2$

Hz, pyridazine), 6.8 (1H, d, $J = 9.2$ Hz, pyridazine), 6.7 (3H, m, phenoxy), 5.5 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$). MS; m/z (DIP): 354 [M^+], 247, 217, 215, 186, 158, 139 (100%).

4.1.8. Synthesis of 1-(4-methylphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9c**)

Yield (19.3%); m.p. 228–229 °C; IR (KBr, cm^{-1}): 3264, 2942, 1692, 1663, 1581, 1525, 1486, 1452; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ_H: 7.8 (2H, d, $J = 8.2$ Hz, phenylethanone), 7.3 (2H, d, $J = 8.2$ Hz, phenylethanone), 7.2 (1H, d, $J = 9.4$ Hz, pyridazine), 7.1 (2H, m, phenoxy), 6.9 (1H, d, $J = 9.4$ Hz, pyridazine), 6.8 (2H, m, phenoxy), 5.6 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$) 2.2 (3H, s, $-CH_3$); MS; m/z (DIP): 334 [M^+], 227, 215, 119 (100%).

4.1.9. Synthesis of 1-(4-tert-butylphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9d**)

Yield (20.6%); m.p. 115 °C; IR (KBr, cm^{-1}): 3300, 2961, 1698, 1661, 1581, 1525, 1487, 1405, 1345, 1277, 1236, 1153; ^{1}H-NMR (CD_3OD) δ_H: 8.0 (2H, d, $J = 8.2$ Hz, di*tert*-butylphenylethanone), 7.56 (2H, d, $J = 8.2$ Hz, di*tert*-butylphenylethanone), 7.3 (1H, d, $J = 9.4$ Hz, pyridazine), 7.1(2H, m, phenoxy), 6.8 (1H, d, $J =$ 9.4 Hz, pyridazine), 6.8 (2H, m, phenoxy), 5.6(2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$), 1.3 (9H, s, $C(CH_3)_3$); MS; m/z (DIP): 376 [M^+], 215, 161 (100%), 146.

4.1.10. Synthesis of 1-(4-fluorophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9e**)

Yield (26.1%); m.p. 204–205 °C; IR (KBr, cm^{-1}): 3283, 2941, 1696, 1662, 1582,1525, 1507, 1487, 1412, 1353, 1280, 1155; ^{1}H-NMR (CD_3OD) δ_H: 8.1 (2H, m, phenylethanone), 7.4 (3H, m, phenylethanone, pyridazine), 7.2 (2H, m, phenoxy), 7.0 (1H, d, $J = 9.2$ Hz, pyridazine), 6.8 (2H, m, phenoxy), 5.4 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$); MS; m/z (DIP): 338 [M^+], 215, 123 (100%).

4.1.11. Synthesis of 1-(3,4-dimethoxyphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9f**)

Yield (33.8%); m.p. 220–221 °C; IR (KBr, cm^{-1}): 3188, 2941, 1659, 1581, 1520, 1484, 1356, 1280, 1255, 1210, 1175, 1136, 1105; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ_H: 9.0 (1H, s, –NH), 7.6 (3H, m, dimethoxy phenylethanone), 7.1 (3H, m, phenoxy, pyridazine), 6.8 (1H, d, $J = 9.4$ Hz, pyridazine), 6.6 (1H, m, phenoxy), 5.5 (2H, s, $-OCH_2$), 3.9 (6H, s, 2*OMe), 3.8 (2H, s, $-CH_2$); MS; m/z (DIP): 380 [M^+], 165 (100%), 149, 137.

4.1.12. Synthesis of 1-(3-methyl-4-chlorophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9g**)

Yield (22.1%); m.p. 232–234 °C; IR (KBr, cm^{-1}): 3428, 2926, 1662,1 1582, 1486, 1347, 1281, 1235, 1153; ^{1}H-NMR (CD_3OD) δ_H: 7.9 (1H, d, $J = 8.6$ Hz, phenylethanone), 7.26 (3H, m, pyridazine, 1H-phenoxy, 1H-phenylethanone), 7.0 (1H, m, phenylethanone), 6.8 (1H, d, $J = 9.4$ Hz, pyridazine), 6.6 (3H, m, phenoxy), 5.5 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$), 2.4 (3H, s, $-CH_3$); MS; m/z (DIP): 368 [M^+], 261, 237, 215, 153 (100%).

4.1.13. Synthesis of 1-(3-methoxy-4-fluorophenyl)-2-[3-(6-oxo-1,6-dihydropyridazinylmethyl)phenoxy]-1-ethanone (**9h**)

Yield (16.42%); m.p. 211–212 °C; IR (KBr, cm^{-1}): 3247, 2941, 1661, 1685, 1608, 1580, 1522, 1488, 1357, 1291, 1240; ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ_H: 7.8 (2H, m, phenylethanone), 7.2 (3H, m, phenylethanone, pyridazine, phenoxy), 6.9 (1H, d, $J = 9.8$ Hz, pyridazine), 6.7 (3H, m, phenoxy), 5.5 (2H, s, $-OCH_2$), 4.1 (3H, s, OCH_3), 3.8 (2H, s, $-CH_2$); MS; m/z (DIP): 368 [M^+], 261, 215, 186, 153 (100%).

4.1.14. Synthesis of 1-(2,4,dichlorophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy]-1-ethanone (**9i**)

Yield (20.7%); m.p. 110–111 °C; IR (KBr, cm^{-1}): 3272, 2927, 1709, 1661, 1581, 1487; ^{1}H-NMR (DMSO-d_6) δ_H: 9.3 (1H, s, pyridazine–NH), 7.89 (2H, m,phenylethanone), 7.2 (1H, m, phenoxy), 7.60 (1H, d, $J = 8.2$ Hz, phenylethanone), 7.35 (1H, d, $J = 9.6$ Hz, pyridazine),7.2 (1H, m), 6.9 (1H, d, $J = 9.6$ Hz, pyridazine), 6.6 (3H, m, phenoxy), 5.6 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$); MS; m/z (DIP): 388 [M^+],353, 325, 316, 281, 270, 230,197, 186, 173 (100%).

4.1.15. Synthesis of N1-(4-methoxy-2-methylphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy] acetamide (**11a**)

Yield (41.27%); m.p. 172–173 °C; IR (KBr, cm^{-1}): 3259, 2924, 1656, 1579, 1548, 1501, 1447, 1390, 1280, 1234, 1156, 1112; ^{1}H-NMR (CD_3OD) δ_H: 7.28 (1H, d, $J = 9.6$ Hz,pyridazine), 7.0 (3H, m, 2-methyl-3-methoxy aniline), 6.8 (1H, d, $J = 9.6$ Hz, pyridazine), 6.7 (4H, m, phenoxy), 4.8 (2H, s, $-OCH_2$), 3.8 (3H, s, OCH_3), 3.7 (2H, s, $-CH_2$), 2.2 (3H, s, $-CH_3$); MS; m/z (DIP): 379 [M^+], 243, 229, 215, 205, 137 (100%).

4.1.16. Synthesis of N1-(3,4,difluorophenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinyl methyl)phenoxy] acetamide (**11b**)

Yield (16.3%); m.p. 118–119 °C; IR (KBr, cm^{-1}): 3282, 2925, 1660, 1588, 1510, 1437, 1336, 1240, 1206, 1155; ^{1}H-NMR (CD_3OD) δ_H: 7.70 (3H, m, difluoro

aniline), 7.2 (3H, m,phenoxy, pyridazine), 6.8 (3H, m, phenoxy, pyridazine), 4.8 (2H, s, $-OCH_2$), 3.3 (2H, s, $-CH_2$); MS; *m*/*z* (DIP): 371 [M^+], 243 (100%), 229, 215, 201, 186, 170, 159.

4.1.17. Synthesis of N1-(4-trifluoromethylphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy] acetamide (**11c**)

Yield (28.7%); m.p. 136–138 °C; IR (KBr, cm^{-1}): 3296, 1653, 1582, 1610, 1549, 1413, 1327, 1260, 1181, 1115, 1080; ^{1}H-NMR (CD_3OD) δ_H: 7.5 (2H, d, $J = 8.6$ Hz, trifluoromethyl phenyl), 7.3 (2H, d, $J = 8.6$ Hz, 4-trifluoromethyl phenyl), 7.2 (1H, d, $J = 9.4$ Hz, pyridazine), 7.0 (2H, m, phenoxy), 6.6 (2H, m, phenoxy), 4.8 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$); MS; *m*/*z* (DIP): 403 [M^+], 257, 243 (100%), 229, 215.

4.1.18. Synthesis of N1-(4-ethylphenyl)-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy] acetamide (**11d**)

Yield (31%); m.p. 94–95 °C; IR (KBr, cm^{-1}): 3291, 2964, 1652, 1581, 1544, 1486, 1454, 1415, 1339, 1309; ^{1}H-NMR (CD_3OD) δ_H: 7.4 (2H, d, $J = 8.4$ Hz, ethyl aniline), 7.2(1H, d, $J = 9.8$ Hz, pyridazine), 7.1 (2H, d, $J = 8.4$ Hz, ethylbenzene), 7.0 (2H, m, phenoxy), 6.8 (1H, d, $J = 9.8$ Hz, pyridazine), 6.6 (2H, m, phenoxy), 4.8 (2H, s, $-OCH_2$), 3.8 (2H, s, $-CH_2$), 2.5 (2H, q, $J = 14$, 9 Hz, $-CH_2$ CH_3), 1.9 (3H, t, $J = 9$ Hz, $-CH_2$ CH_3); MS; *m*/*z* (DIP): 363 [M^+], 257, 243, 215, 202 121 (100%).

4.1.19. Synthesis of N1-benzo[d][1,3]dioxo[5-yl-2-[3-(6-oxo-1,6-dihydro-3-pyridazinylmethyl)phenoxy] acetamide (**11e**)

Yield (17.7%); m.p. 219–220 °C; IR (KBr, cm^{-1}): 3249, 2942, 1681, 1574, 1502, 1450, 1390; ^{1}H-NMR (DMSO-d_6) δ_H: 10.2 (1H, s, –NH–CO), 9.4 (1H, s, –NH, pyridazine), 7.33 (3H, m, methylene dioxy aniline), 7.1 (1H, m, phenoxy), 7.0 (2H, m, pyridazine, phenoxy), 6.9 (3H, m, phenoxy, pyridazine) 5.9 (2H, s, $-O-CH_2-O$), 5.8 (2H, s, $-CH_2$), 4.8 (2H, s, $-OCH_2$); MS; *m*/*z* (DIP): 379 [M^+], 243, 215, 176 137 (100%).

Table 2
Carrageenan-induced rat paw edema [a]

Compound	Dose (mg kg^{-1})	% Inhibition
11b	30	32
11c	30	8
11d	30	0
Rofecoxib	3	65
Celecoxib	10	59

ED_{50} values: rofecoxib, 3 mg kg^{-1}; celecoxib, 7.9 mg kg^{-1}.
[a] Wistar male/female, $n = 5$, body weight: 120–140 g, oral administration vehicle = 0.25% CMC.

4.2. Pharmacology

4.2.1. Spectrophotometric assay of COX-1 and COX-2

Microsomal fractions of Ram seminal vesicles were used as a source of COX-1 enzyme [18] and microsomes from Sf9 cells infected with baculo virus containing human COX-2 cDNA was used as a source of COX-2 enzyme [19]. Enzyme activity was measured using a chromogenic assay based on the oxidation of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2 as per the procedure described by Copeland et al. [20] with the following modifications. The assay mixture (1000 μL) contained 100 μM Tris pH 8.0, 3 μM EDTA, 15 μM hematin, 150 units enzyme and 8% DMSO. The mixture was pre-incubated at 25 °C for 15 min before initiation of enzyme reaction in presence of compound/vehicle. The reaction was initiated by the addition of 10 μM arachidonic acid and 120 μM TMPD. The enzyme activity was measured by estimation of the reaction followed from increase in absorbance at 603 nm.

4.2.2. Carrageenan-induced rat paw edema assay

Male/female Wistar rats (120–140 g) were fasted for 16 h before the experiment. Compounds were suspended in 0.25% carboxy methylcellulose and administered orally in a volume of 10 mL kg^{-1} 2 h before carrageenan injection. Edema was induced in rats by intradermal injection of 50 μL of 1% λ-carrageenan in saline into the plantar surface of the right hind paw [21]. The paw volume was monitored before and 3 h after carrageenan injection using plethysmometer (Ugo-Basile, Italy). The paw edema was compared with the vehicle treated group and the percent inhibition was calculated (Table 2).

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