Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi, India

Synthesis of some new 2-(2-fluoro-4-biphenylyl)propionic acid derivatives as potential anti-inflammatory agents

M. Amir, S. Kumar

Received March 1, 2004, accepted May 20, 2004

Dr. Mohammad Amir, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India
mamir_s2003@yahoo.co.in

Pharmazie 60: 175–180 (2005)

The synthesis of a group of 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,2,4-triazine derived from 2-(2-fluoro-4-biphenylyl) propionic acid is described. The structures of new compounds are supported by IR, ^{1}H NMR and MS data. These compounds were tested *in vivo* for their anti-inflammatory activity. The compounds which showed activity comparable to the standard drug flurbiprofen, were screened for their analgesic, ulcerogenic and lipid peroxidation activities. Five out of seventeen new compounds, showed very good anti-inflammatory activity in the carrageenan induced rat paw edema test with negligible ulcerogenic action. The compounds, which showed less ulcerogenic action, also showed reduced malondialdehyde production (MDA), which is one of the byproducts of lipid peroxidation. The study showed that the compounds inhibit the induction of gastric mucosal lesions and it can be suggested from our results that their protective effects may be related to an inhibition of lipid peroxidation in the gastric mucosa.

1. Introduction

The gastrointestinal (GI) side effects of non steroidal anti-inflammatory drugs (NSAIDs) are generally attributed to two factors, local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis (Smith et al. 1998; Hawkey et al. 2000). The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the cyclooxygenase enzymes (COXs) (Smith et al. 1998; Warner et al. 1999). Recently, it was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently (Marnett and Kalgutkar 1998, 1999). COX-1 provides cytoprotection in the GI tract whereas inducible COX-2 mediates inflammation (Parsit and Reindeau 1997; Almansa et al. 2003). Since most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2 (Jackson and Hawkey 1999), chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration (Lanza 1998).

Synthetic approaches based upon NSAIDs chemical modification have been undertaken in order to improve NSAID safety profile. Studies describe the derivatization of the carboxylate function (Kalgutkar et al. 2000; Duflos et al. 2001; Kalgutkar et al. 1998) of representative NSAIDs, which resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect. Furthermore, certain compounds bearing the 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus have been reported to have significant anti-inflammatory activity (Mullican et al. 1993; Omar et al. 1996; Amir et al. 1999; Tozkoparan et al. 2000; Palaska et al. 2002). In our attempt to discover new and useful agents for the treatment of inflammatory diseases we have replaced the carboxylic acid group of flurbiprofen with additional heterocycles, which have been found to possess an interesting profile of anti-inflammatory activity with significant reduction in their ulcerogenic effect. The heterocycles reported here are 1,3,4-oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,2,4-triazine.

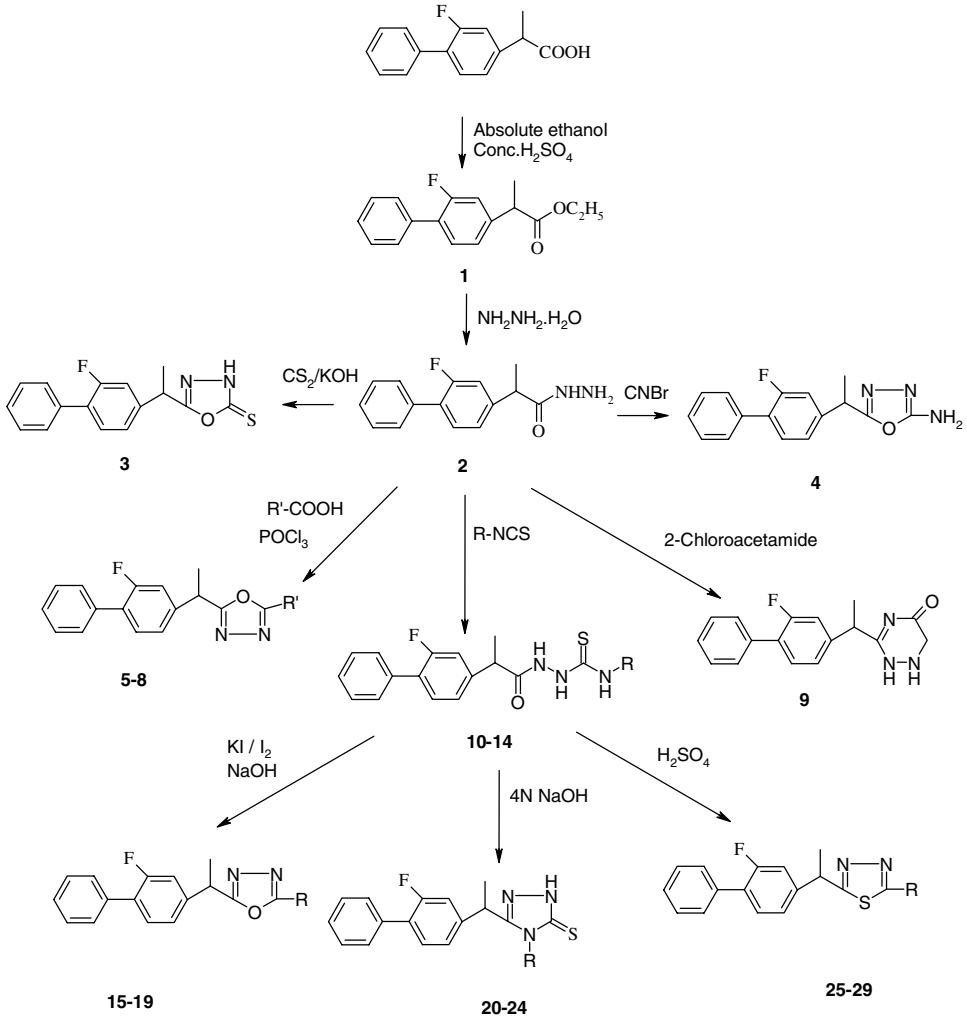
2. Investigations, results and discussion

2.1. Synthesis of compounds 1–29

The acid hydrazide **2** was prepared by esterification of 2-(2-fluoro-4-biphenylyl) propionic acid followed by treatment with hydrazine hydrate in absolute ethanol. The reaction of hydrazide **2** with carbon disulphide in alkaline medium afforded, after acidic treatment, 5-[2-(2-fluoro-4-biphenylyl)ethyl]-2-mercapto-1,3,4-oxadiazole (**3**). Treatment of hydrazide with cyanogen bromide afforded 5-[2-(2-fluoro-4-biphenylyl)ethyl]-2-amino-1,3,4-oxadiazole **4**. Various 2-aryl-1,3,4-oxadiazoles **5**–**8** were prepared by treatment of hydrazide with appropriate aromatic acids in the presence of phosphorus oxychloride. 3-[2-(2-Fluoro-4-biphenylyl)ethyl]-1,2,5,6-tetrahydro-1,2,4-triazine-5-one **9** was prepared by condensation of hydrazide with chloroacetamide (Scheme).

Furthermore, hydrazide **2** on treatment with various alkyl/aryl isothiocyanate gave N^1-[2-(2-fluoro-4-biphenylyl)propionyl]-N^4-alkyl/arylthiosemicarbazides **10**–**14**. The thiosemicarbazides were oxidatively cyclised to 2-alkyl/arylamino-5-substituted-1,3,4-oxadiazoles **15**–**19** by elimina-

Scheme



tion of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. On heating with 4N-NaOH in ethanol the thiosemicarbazides **10**–**14** underwent smooth cyclisation through dehydration to afford the 5-substituted-4-alkyl/aryl-3-mercapto-4*H*-1,2,4-triazoles **20**–**24**. 2-Alkyl/arylamino-5-substituted–1,3,4-thiadiazoles **25**–**29** were obtained by cyclisation of **10**–**14** by treating with polyphosphoric acid.

The structures of the various compounds synthesized were assigned on the basis of elemental analysis as well as IR, ^{1}H NMR and MS data. Physical data for the compounds are given in Table 1.

2.2. Biological studies

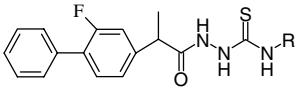
2.2.1. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds **3, 4, 7**–**9, 15**–**21, 23**–**27** were evaluated by the carrageenan induced paw edema method of Winter et al. (1962). The compounds were tested at 10 mg/kg oral dose and were compared with the standard drug flurbiprofen. The tested compounds showed anti-inflammatory activity ranging from 66.07 to 94.11% inhibition (Table 2), whereas the standard drug flurbiprofen showed 95.57% inhibition after 4 h. The anti-inflammatory activity of 1,3,4-oxadiazole derivatives was in the range from 70.25% to 94.11%. The oxadiazole derivative (**15**) having an *n*-butyl amino group showed the maximum activity, whereas when this group was replaced by a cyclohexyl amino group (**16**) the activity was found to be minimum. Replacement of the *n*-butyl group by an aryl amino group at 2nd position of the oxadiazole nucleus resulted in the decrease of activity. When aryl amino was replaced by 2-mercapto (**3**), 2-amino (**4**), 2,4-dichlorophenyl methoxy (**7**) and *p*-aminophenyl groups (**8**), the activity was found to be reduced.

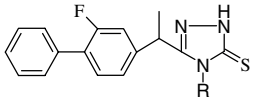
The 1,2,4-triazole derivatives of flurbiprofen showed anti-inflammatory activity ranging from 66.07% to 90.58%. The highest activity was found in the triazole derivative (**20**) having an *n*-butyl group at 4th position, whereas when this group was replaced by cyclohexyl group (**21**), the activity was found to be minimum. The replacement of the *n*-butyl group by *p*-methylphenyl (**24**) and *p*-fluorophenyl (**23**) groups also resulted in decreased activity.

The 1,3,4-thiadiazole derivatives of flurbiprofen showed anti-inflammatory activity ranging from 74.70% to 92.35%. The maximum activity was shown by the thiadiazole derivative (**25**) having a *n*-butyl amino group at 2nd position. On replacing this group with *p*-chlorophenyl amino group (**27**), the activity was found to be reduced. The thiadiazole derivative having a cyclohexyl amino group (**26**) at the 2nd position of the thiadiazole nucleus showed minimum activity.

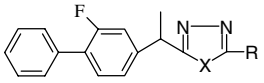
Table 1: Physical data of 2-(2-fluoro-4-biphenylyl)propionic acid derivatives



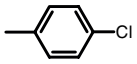
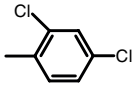
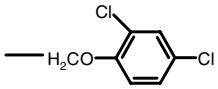
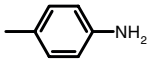
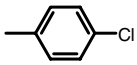
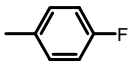
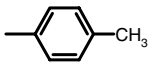
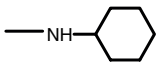
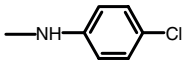
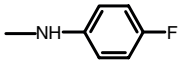
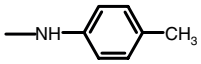
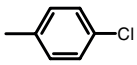
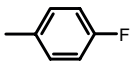
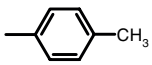
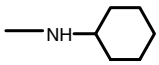
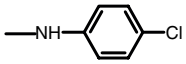
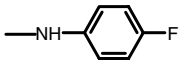
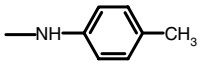
10-14



20-24



X = O (**3-8, 15-19**); S (**25-29**)

Compd.	R	Yield (%)	M.P. (°C)	Mol.formula	Mol.Wt.
3	−SH	72	322	$C_{16}H_{13}N_2OSF$	300.36
4	$-NH_2$	70	310	$C_{16}H_{14}N_3OF$	283.31
5		68	245	$C_{22}H_{16}N_2OClF$	378.84
6		64	90	$C_{22}H_{15}N_2OCl_2F$	413.28
7		75	120	$C_{23}H_{17}N_2O_2Cl_2F$	443.31
8		66	190	$C_{22}H_{18}N_3OF$	359.41
10	$-CH_2-CH_2-CH_2-CH_3$	64	116	$C_{20}H_{24}N_3OSF$	373.50
11		56	180	$C_{22}H_{26}N_3OSF$	399.53
12		62	178–180	$C_{22}H_{19}N_3OSClF$	427.93
13		63	182–184	$C_{22}H_{19}N_3OSF_2$	411.48
14		67	158	$C_{23}H_{22}N_3OSF$	407.51
15	$-NH-CH_2-CH_2-CH_2-CH_3$	68	138–140	$C_{20}H_{22}N_3OF$	339.42
16		60	300	$C_{22}H_{24}N_3OF$	365.45
17		67	160–162	$C_{22}H_{17}N_3OClF$	393.85
18		59	>300	$C_{22}H_{17}N_3OF_2$	377.40
19		47	>300	$C_{23}H_{20}N_3OF$	373.43
20	$-CH_2-CH_2-CH_2-CH_3$	61	156–158	$C_{20}H_{22}N_3SF$	355.48
21		78	60	$C_{22}H_{24}N_3SF$	381.52
22		55	170–172	$C_{22}H_{17}N_3SClF$	409.92
23		60	200–204	$C_{22}H_{17}N_3SF_2$	393.46
24		42	280	$C_{23}H_{20}N_3SF$	389.50
25	$-NH-CH_2-CH_2-CH_2-CH_3$	46	42–44	$C_{20}H_{22}N_3SF$	355.48
26		54	semisolid	$C_{22}H_{24}N_3SF$	381.52
27		63	54–56	$C_{22}H_{17}N_3SClF$	409.92
28		68	semisolid	$C_{22}H_{17}N_3SF_2$	393.46
29		71	48–50	$C_{23}H_{20}N_3SF$	389.50

Satisfactory analysis for C,H,N was obtained for all the compounds within ± 0.4% of the theoretical values

When the hydrazide of flurbiprofen was treated with 2-chloroacetamide, a 1,2,4-triazine derivative was obtained, which also showed significant anti-inflammatory activity.

2.2.2. Analgesic activity

The compounds, which showed anti-inflammtory activity close to standard drug flurbiprofen, were tested for analgesic activity. The compounds **9**, **15**, **19**, **20**, **25** showed analgesic activity ranging from 18.13% to 84.06% inhibition, whereas the standard drug flurbiprofen showed 51.09% at 10 mg/kg oral dose. Among all the tested compounds, oxadiazoles **15**, **19** showed maximum activity in comparison to thiadiazole and triazole derivatives. Triazole derivative **20** showed minimum activity, whereas the triazine derivative **9** showed the highest activity, which is far better than the standard compound.

2.2.3. Acute ulcerogenesis

The compounds, which were screened for analgesic activity, were further screened for their ulcerogenic activity. All the compounds were tested at 30 mg/kg oral dose.
The tested compounds showed a significant reduction in ulcerogenic activity with a severity index ranging from 0.250 ± 0.11 to 1.666 ± 0.17, whereas the standard drug flurbiprofen showed high a severity index of 3.833 ± 0.17. The maximum reduction in ulcerogenic activity was found with the 1,3,4-thiadiazole derivative having a *n*-butylamino group (**25**), whereas the oxadiazole derivative having a *n*-butylamino group (**15**) showed minimum reduction in ulcerogenic activity. The other compounds showed moderate reduction in severity index.

2.2.4. Lipid peroxidation

It has been reported that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) tissue content, a byproduct of lipid peroxidation (Naito et al. 1998; Pohle et al. 2001). Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation.
The lipid peroxidation was measured in nmol of MDA/100 mg of tissue. Flurbiprofen (standard drug) showed the maximum MDA values (9.983 ± 0.08), whereas the control group showed 3.370 ± 0.01 nmol/100 mg tissue. It was found that all the cyclised derivatives with less ulcerogenic activity also showed a reduced lipid peroxidation (Table 2). Thus, these studies show that the compounds synthesized have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.
From these studies compound **25**, a thiadiazole derivative, has emerged as the lead compound, which showed maximum reduction in ulcerogenic activity followed by triazine, oxadiazole and triazole derivatives.

3. Experimental

Melting points were measured in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FT-IR spectrometer (ν_{max} in cm^{-1}) and ^1H NMR spectra on a Bruker DRX-300 (300 MHz FT NMR) spectrophotometer using TMS as internal reference (Chemical shift in δ, ppm). Mass spectra were recorded at Jeol SX-102 (FAB) spectrometer. Purity of the compounds was checked on silica gel G plates using iodine vapours as visualising agent. 2-(2-Fluoro-4-biphenylyl)propionic acid (flurbiprofen) was received from Sunways Pvt. Ltd, Mumbai, India as a gift sample.

3.1. Chemistry

3.1.1. Ethyl-[2-(2-fluoro-4-biphenylyl)propionate] (**1**)

The compound was prepared by a procedure given in the literature (Furniss et al. 1998).

3.1.2. 2-(2-Fluoro-4-biphenylyl)propionic acid hydrazide (**2**)

Compound **1** (10 mmol) and hydrazine hydrate (20 mmol) were refluxed in absolute ethanol (50 ml) for 18 h. The mixture was concentrated, cooled and poured in crushed ice in small portions while stirring and kept for 3–4 h at room temperature. The solid thus separated out was filtered, dried and crystallized from ethanol, m.p. 96 °C, yield 72%. 258.30. ^1H NMR ($CDCl_3$): 1.46 (d, J = 7 Hz, 3 H, CH_3), 3.68 (q, J = 7 Hz, 1 H, CH), 4.50 (s, 2 H, NH_2), 7.03–7.40 (m, 8 H, ArH), 8.21 (s, 1 H, CONH). Mass spec-

Table 2: Biological data of flurbiprofen derivatives

Compound	Anti-inflammatory activity (% Inhibition ± S.E.M)	Analgesic activity (% Inhibition ± S.E.M)	Ulcerogenic activity (Severity index ± S.E.M)	nmol MDA content ± S.E.M)/ 100 mg tissue
Control	–	–	0.000 ± 0.00	3.371 ± 0.01
Flurbiprofen	95.57 ± 0.64 [a]	51.09 ± 0.85 [a]	3.833 ± 0.17	9.983 ± 0.08
3	74.70 ± 3.62 [a]	–	–	–
4	76.47 ± 5.26 [a]	–	–	–
7	76.47 ± 4.29 [a]	–	–	–
8	74.70 ± 1.96 [a]	–	–	–
9	85.96 ± 2.92 [a]	84.06 ± 0.85 [a]	0.583 ± 0.20 [a]	4.428 ± 0.12 [a]
15	94.11 ± 2.63 [a]	45.05 ± 1.00 [a]	1.666 ± 0.17 [a]	6.341 ± 0.06 [a]
16	70.25 ± 3.41 [a]	–	–	–
17	82.35 ± 2.63 [a]	–	–	–
18	73.84 ± 3.50 [a]	–	–	–
19	88.20 ± 0.00 [a]	34.61 ± 0.85 [a]	0.333 ± 0.11 [a]	4.252 ± 0.10 [a]
20	90.58 ± 1.96 [a]	18.13 ± 0.85 [a]	1.416 ± 0.17 [a]	4.855 ± 0.12 [a]
21	66.07 ± 1.53 [a]	–	–	–
23	79.76 ± 1.99 [a]	–	–	–
24	83.92 ± 0.80 [a]	–	–	–
25	92.35 ± 2.48 [a]	21.42 ± 0.58 [a]	0.250 ± 0.11 [a]	4.065 ± 0.08 [a]
26	74.70 ± 1.96 [a]	–	–	–
27	87.88 ± 1.84 [a]	–	–	–

Anti-inflammatory and analgesic activities of the test compounds were compared w.r.t. control. Ulcerogenic and lipid peroxidation were compared w.r.t. standard drug i.e. flurbiprofen.
[a] $P < 0.0001$; Data were analyzed by student's *t*-test for $n = 6$.

tra of the compound exhibited molecular ion peak at m/z 258 (M^+), other important fragments were observed at 259 (M^++1), 227, 199, 185, 166.
$C_{15}H_{15}N_2OF$

3.1.3. 5-[2-(2-Fluoro-4-biphenylyl) ethyl]-2-mercapto-1,3,4-oxadiazole (**3**)

A mixture of 1.30 g (5 mmol) **2**, KOH (5 mmol) and carbondisulphide (5 ml) in ethanol (50 ml) was refluxed on a steam bath for 10 h. The solution was then concentrated, cooled and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried and crystallized from ethanol (Table 1). The IR spectrum of the compound showed bands at 2930 (N−H), 1612 (C=N), 1160 (C=S). ^{1}H NMR ($CDCl_3$): 1.73 (d, J = 7 Hz, 3 H, CH_3), 4.20 (q, J = 7 Hz, 1 H, CH), 7.08–7.51 (m, 8 H, ArH), 10.34 (br. s, 1 H, NH).

3.1.4. 5-[2-(2-Fluoro-4-biphenylyl) ethyl]2-amino-1,3,4-oxadiazole (**4**)

To an ethanolic solution of 0.26 g (1 mmol) **2**, cyanogen bromide (1 mmol) was added. The reaction mixture was stirred with heating at 55–60 °C for 3 h. The resulting solution was cooled and neutralized with sodium bicarbonate solution. The solid thus separated out was filtered, washed with water, dried and crystallized from methanol (Table 1). The IR spectrum of the compound showed bands at 3296 (N−H), 2985 (C−H), 1655 (C=N). ^{1}H NMR ($CDCl_3$): 1.68 (d, J = 7 Hz, 3 H, CH_3), 4.23 (q, J = 7 Hz, 1 H, CH), 6.58 (s, 2 H, NH_2), 7.08–7.84 (m, 8 H, ArH).

3.1.5. General procedure for the synthesis of 5-[2-(2-Fluoro-4-biphenylyl) ethyl]-2-aryl-1,3,4-oxadiazoles (**5–8**)

Compound **2** (0.26 g, 1 mmol) and the appropriate aromatic acid (1 mmol) was dissolved in phosphorus oxychloride and refluxed for 20–24 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, washed with water, dried and crystallized from ethanol (Table 1). IR spectra of the compound **5–8** showed bands at 2982–2918 (C−H) and 1655–1650 (C=N). In case of **8**, there was an additional N−H stretching at 3464 cm^{-1}. ^{1}H NMR ($CDCl_3$) **7**: 1.55 (d, J = 7.2 Hz, 3 H, CH_3), 3.79 (q, J = 7.2 Hz, 1 H, CH), 4.66 (s, 2 H, CH_2O), 7.12–7.53 (m, 11 H, ArH). The MS of compound **7** exhibited a molecular ion peak at m/z 443 (M^+), other important fragments were observed at 442, 445 (M^++2), 447 (M^++4), 244, 227, 225, 199, 185, 166.

3.1.6. 3-[2-(2-Fluoro-4-biphenylyl) ethyl]-1,2,5,6-tetrahydro-1,2,4-triazin-5-one (**9**)

To 2.60 g (10 mmol) of compound **2** chloroacetamide (10 mmol) and dimethyl formamide (80 ml) were added and the reaction mixture was refluxed for 25 h. It was then concentrated and cooled, whereupon a solid separated out, which was filtered, washed with ethanol and crystallized from DMF/water, m.p. 164–166 °C, yield 37%. The IR spectrum of the compound showed bands at 3380 (N−H), 2932 (C−H), 1656 (C=O), 1581 (C=N). ^{1}H NMR ($CDCl_3$): 1.56 (d, J = 7 Hz, 3 H, CH_3), 2.98 (s, 2 H, CH_2 of triazinone), 3.67 (q, J = 7 Hz, 1 H, CH), 5.48 (br. s, 1 H, NH), 5.93 (br. s, 1 H, NH), 7.16–7.42 (m, 8 H, ArH). The MS of compound **9** exhibited a molecular ion peak at m/z 298 (M^++1), other fragments were observed at 225, 199, 185.
$C_{17}H_{16}N_3OF$ (297.3)

3.1.7. General procedure for the synthesis of N^1-[2-(2-fluoro-4-biphenylyl)-propionyl]N^4-alkyl/aryl-thiosemicarbazides **10–14**

A mixture of 2.60 g (10 mmol) **2**, alkyl/aryl isothiocyanate (10 mmol) and ethanol (50 ml) was refluxed on steam bath for 6 h. It was then concentrated, cooled and kept overnight in refrigerator. The solid thus separated out, was filtered, washed with ethanol, dried and crystallized from ethanol (Table 1). IR spectra of the compounds **10–14** showed bands at 3368–3144 (N−H), 2930–2853 (C−H), 1680–1660 (C=O), 1178–1150 (C=S). ^{1}H NMR ($CDCl_3$) **10**: 0.86 (t, J = 7.2 Hz, 3 H, CH_3), 1.26–1.31 (m, 2 H, $CH_3C\underline{H}_2$), 1.44–1.46 (m, 2 H, $CH_3CH_2C\underline{H}_2$), 1.59 (d, J = 7 Hz, 3 H, CH_3), 3.48–3.50 (m, 2 H, $NHC\underline{H}_2$), 3.76 (q, J = 7 Hz, 1 H, CH), 6.76 (t, J = 7.2 Hz, 1 H, NH), 7.10–7.53 (m, 8 H, ArH), 8.98 (s, 1 H, CSNH), 9.09 (s, 1 H, CONH). ^{1}H NMR ($CDCl_3$) **12**: 1.61 (d, J = 6.6 Hz, 3 H, CH_3), 3.78 (q, J = 6.6 Hz, 1 H, CH), 7.13–7.62 (m, 13 H, 12 ArH & 1 NH), 8.60 (s, 1 H, CSNH), 9.67 (s, 1 H, CONH). The MS of compound **10** exhibited molecular ion peak at m/z 374 (M^++1), other important fragments were observed at m/z 355, 326, 258, 227, 199.

3.1.8. General procedure for the synthesis of 5-[2-(2-Fluoro-4-biphenylyl) ethyl]-2-alkyl/aryl amino-1,3,4-oxadiazoles **15–19**

A suspension of **10–14** (2 mmol) in ethanol (50 ml) was dissolved in aqueous sodium hydroxide (5 N, 1 ml) with cooling and stirring, resulting of a clear solution. To this, iodine in potassium iodide solution (5%) was added gradually with stirring until the colour of iodine persisted at room temperature. The reaction mixture was then refluxed for 1–4 h on a water bath. It was then cooled and poured over crushed ice. The solid mass that separated out was filtered, dried and crystallized from ethanol (Table 1). IR spectra of the compounds **15–19** showed bands at 3462–3426 (N−H), 2936–2924 (C−H), 1580–1556 (C=N). ^{1}H NMR ($CDCl_3$) **15**: 1.34 (t, J = 7 Hz , 3 H, CH_3), 1.74–1.77 (m, 2 H, $CH_3C\underline{H}_2$), 2.19–2.27 (m, 2 H, $CH_3CH_2C\underline{H}_2$), 2.32 (d, J = 7 Hz, 3 H, CH_3), 4.12–4.19 (m, 2 H, $NHC\underline{H}_2$), 4.32 (q, J = 7 Hz, 1 H, $C\underline{H}CH_3$), 7.51–7.92 (m, 9 H, 8 ArH & 1 NH). ^{1}H NMR (DMSO-d_6) **16**: 1.26–1.65 (m, 11 H, cyclohexyl), 1.87 (d, J = 7 Hz, 3 H, CH_3), 4.63 (q, J = 7 Hz, 1 H, CH), 7.17–7.50 (m, 8 H, ArH), 8.61 (s, 1 H, NH). ^{1}H NMR ($CDCl_3$) **17**: 1.36 (d, J = 7 Hz, 3 H, CH_3), 4.76 (q, J = 7 Hz, 1 H, CH), 7.35–7.99 (m, 13 H, 12 ArH & 1 NH).

3.1.9. General procedure for the synthesis of 5-[(2-Fluoro-4-biphenylyl) ethyl]-4-alkyl/aryl-3-mercapto-1,2,4(H)-triazoles **20–24**

A suspension of **10–14** (2 mmol) in ethanol (25 ml) was dissolved in aqueous sodium hydroxide (4 N, 2 ml) and gently refluxed for 6–8 h. The resulting solution was concentrated, cooled and filtered. The filtrate was adjusted to pH 5–6 with dilute acetic acid and was kept aside for 1 h. The crystals produced were filtered, washed with water, dried and recrystallized from ethanol (Table 1). IR spectra of the compounds **20–24** showed bands at 2971–2910 (C−H), 1576–1560 (C = N), 1097–1060 (C = S). ^{1}H NMR ($CDCl_3$) **20**: 0.81 (t, J = 6.6 Hz, 3 H, CH_3), 1.21–1.25 (m, 2 H, $CH_3-C\underline{H}_2$), 1.68–1.71 (m, 2 H, $CH_3CH_2C\underline{H}_2$), 1.82 (d, J = 7 Hz, 3 H, $C\underline{H}_3$-CH), 3.87 (t, J = 7 Hz, 2 H, $N-C\underline{H}_2$), 4.05 (q, J = 7 Hz, 1 H, $C\underline{H}-CH_3$), 6.97–7.45 (m, 8 H, ArH), 11.37 (br. s, 1 H, NH). The MS of compound **20** exhibited a molecular ion peak at m/z 355 (M^+), other important fragments were found at 356 (M^++ 1), 322, 299, 296, 199, 185. The MS of compound **21** exhibited a molecular ion peak at m/z 381 (M^+), other important fragments were found at 348, 329, 268, 199, 185. ^{1}H NMR ($CDCl_3$) **22**: 1.73 (d, J = 7 Hz, 3 H, CH_3), 4.34 (q, J = 7 Hz, 1 H, CH), 7.19–7.96 (m, 12 H, ArH), 11.84 (br. s, 1 H, NH). ^{1}H NMR ($CDCl_3$) **23**: 1.79 (d, J = 7 Hz, 3 H, CH_3), 3.84 (q, J = 7 Hz, 1 H, CH), 6.69–7.55 (m, 12 H, ArH), 8.25 (s, 1 H, NH). ^{1}H NMR ($CDCl_3$) **24**: 1.63 (d, J = 7 Hz, 3 H, CH_3), 2.40 (s, 3 H, CH_3), 3.85 (q, J = 7 Hz, 1 H, CH), 6.68–7.60 (m, 12 H, ArH), 8.23 (s, 1 H, NH).

3.1.10. General procedure for the synthesis of 5-[(2-fluoro-4-biphenylyl) ethyl]-2-alkyl/arylamino-1,3,4-thiadiazoles **25–29**

The thiosemicarbazides **10–14** (2 mmol) was added gradually with stirring to polyphosphoric acid (12–15 ml) at 120 °C for 20 min. The reaction mixture was heated with stirring for another 2–3 h and poured over crushed ice. The precipitated mass was filtered, washed with water and crystallized from methanol (Table 1). The IR spectra of compounds **25–29** showed bands at 3424–3402 (N−H), 2930–2912 (C−H), 1622–1612 (C=N). ^{1}H NMR (DMSO-d_6) **27**: 1.66 (d, J = 7 Hz, 3 H, CH_3), 4.57 (q, J = 7 Hz, 1 H, CH), 7.02–7.57 (m, 13 H, 12 ArH & 1 NH). ^{1}H NMR (DMSO-d_6) **29**: 1.69 (d, J = 7 Hz, 3 H, $CH-C\underline{H}_3$), 2.24 (s, 3 H, CH_3), 4.58 (q, J = 7 Hz, 1 H, CH), 7.10–7.54 (m, 13 H, 12 ArH & 1 NH).

3.2. Biological evaluation

The experiments were performed on albino rats of Wistar strain of either sex, weighing 180–200 g. The animals were maintained at 25 ± 2 °C, 50 ± 5% relative humidity, 12 h light/dark cycle. Food and water were freely available upto the time of experiments. The test compounds were dissolved in 1% carboxy methyl cellulose (CMC) solution.

3.2.1. Anti-inflammatory activity

This test was performed by the following procedure of Winter et al. (1962) on groups of six animals each. A freshly prepared suspension of carrageenan (1.0% w/v, 0.1 ml) was injected in the plantar 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 suspended in 1.0% CMC given orally 1 h before the carrageenan treatment. The volume was measured before and after 4 h of carrageenan treatment by means of a pleythysmometer. The percent anti-inflammatory activity was calculated according to the formula:

$$\%\text{Anti-inflammatory activity} = (V_c - V_t/V_c) \times 100 \quad (1)$$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.
Data are expressed as mean ± S.E.M., the student's *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

3.2.2. Analgesic activity

The acetic acid induced writhing test (Koster et al. 1959) was performed by an i.p. injection of 1% aqueous acetic acid solution in a volume of 0.1 ml. In each group six albino mice were kept. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min.

Screening of analgesic activity was performed after p.o. administration of test drugs at the dose of 10 mg/kg. The compounds, which exhibited good anti-inflammatory activity comparable to that of flurbiprofen, were screened for analgesic activity. All compounds were dissolved in 1% CMC solution. One group was kept as control and received p.o. administration of 1% CMC. Flurbiprofen was used as reference drug. 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 % inhibition.

$$\%\text{Analgesic activity} = (n - n'/n) \times 100 \quad (2)$$

where n = mean number of writhes of control group, n′ = mean number of writhes of test group.

Data are expressed as mean ± S.E.M., the student's *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

3.2.3. Acute ulcerogenesis

Acute ulcerogenesis was determined according to Cioli et al. (1979). Albino rats were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or flurbiprofen at the dose of 30 mg/kg. Control rats received p.o. 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 a normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in 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.

Data are expressed as mean ± S.E.M., the student's *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the test compounds.

3.2.4. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. (1979). After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 ml of 1.15% ice cold KCl solution. The homogenate was supplemented with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of acetate buffer (pH-3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min. After cooling, the reactants were supplemented with 5 ml of the mixture of *n*-butanol : pyridine (15 : 1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using extinction coefficient $1.56 \times 10^5\ cm^{-1}\ M^{-1}$.

Data are expressed as mean ± S.E.M., the student's *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the test compounds.

Acknowledgements: The authors are thankful to head of department, pharmaceutical chemistry for providing laboratory facilities and Central Drug Research Institute (C.D.R.I) for spectral analysis of the compounds. Authors are also thankful to Mrs. Shaukat Shah, in-charge animal house for providing Wistar strain rats and Swiss albino mice for pharmacological studies. One of the author (KS) is grateful to the University Grants Commission (U.G.C) New Delhi, for providing financial assistance.

References

Almansa C, Alfon J, de Arriba, Cavalcanti FL, Escamilla I, Gomez LA, Miralles A, Soliva R, Bartroli J, Carceller E, Merlos M, Rafanell JG (2003) Synthesis and structure activity relationship of a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. J Med Chem 46: 3463–3475.

Amir M, Oberoi A, Alam S (1999) Synthesis and anti-inflammatory activity of some new 6-methoxy-α-methyl-2-naphthalene acetic acid derivatives. Indian J Chem 38B: 237–239.

Cioli V, Putzolu S, Rossi V, Sorza Barcellona P, Corradino C (1979) The role of direct tissue contact in the production of gastro-intestinal ulcers by anti-inflammatory drugs in rats. Toxicol Appl Pharmacol 50: 283–289.

Duflos M, Nourrisson MR, Brelet J, Courant J, Le Baut G, Grimaud N, Petit JY (2001) N-Pyridinyl-indole-3-(alkyl) carboxamides and derivatives as potential systemic and topical inflammation inhibitors. Eur J Med Chem 36: 545–553.

Furniss B, Hannaford AH, Smith PWG, Tatchell AR (1998) Vogel's Text book of practical organic chemistry, 5th ed., Addison Wesley Longman, Inc., p. 1077.

Hawkey C, Laine L, Simon T, Beaulieu A, Maldonado-Cocco J, Acevedo E, Shahane A, Quan H, Bolognese J, Mortensen E (2000) Comparison of the effect of rofecoxib, ibuprofen and placebo on the gastroduodenal mucosa of patients with osteoarthritis: A randomized, double blind, placebo-controlled trial. Arthritis Rheum 43: 370–377.

Jackson LM, Hawkey CJ (1999) Gastro-intestinal effects of COX-2 inhibitors. Exp Opin Invest Drugs 8: 963–971.

Kalgutkar AS, Crews BC, Rowlinson SW, Garner C, Seibert K, Marnett LJ (1998) Aspirin like molecules that covalently inactivate cyclo-oxygenase-2. Science 280: 1268–1270.

Kalgutkar AS, Marnett AB, Crews BC, Remmel RP, Marnett LJ (2000) Ester and amide derivatives of the non-steroidal anti-inflammatory drugs, indomethacin, as selective cyclo-oxygenase-2 inhibitor. J Med Chem 43: 2860–2870.

Koster R, Anderson M, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18: 412.

Lanza FL (1998) A guideline for the treatment and prevention of NSAID-induced ulcers. Am J Gastroenterol 93: 2037–2046.

Marnett LJ, Kalgutkar AS (1998) Design of selective inhibitors of cyclo-oxygenase-2 as non-ulcerogenic anti-inflammatory activity. Curr Opin Chem Biol 2: 482–490.

Marnett LJ, Kalgutkar AS (1999) Cyclo-oxygenase-2 inhibitors: Discovery, selectivity and the future. Trends Pharmacol Sci 20: 465–469.

Mullican MD, Wilson MW, Connor DT, Kostlan CR, Shrier DJ, Dyer RD (1993) Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, -1,3,4-oxadiazoles, and -1,2,4-triazoles as orally-active, non-ulcerogenic anti-inflammatory agents. J Med Chem 36: 1090–1099.

Naito Y, Yoshikawa T, Yoshida N, Kondo M. (1998) Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric mucosal injury. Dig Dis Sci 43: 30s–34s.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Anal Biochem 95: 351–358.

Omar FA, Mahfouz NM, Rahman MA (1996) Design, synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives. Eur J Med Chem 31: 819–825.

Palaska E, Sahin G, Kelicen P, Durlu NT, Altinok G (2002) Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazide; 1,3,4-oxadiazoles; 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. Farmaco 57: 101–107.

Parsit P, Reindeau D (1997) Selective cyclo-oxygenase-2 inhibitors. Annu Rep Med Chem 32: 211–220.

Pohle T, Brzozowski T, Becker JC, Vander Voort IR, Markmann A, Konturek SJ, Moniczewski A, Domschke W, Konturek JW (2001) Role of reactive oxygen metabolites in aspirin-induced gastric damage in humans, gastroprotection by vitamin. Aliment Pharmacol Ther 15: 677–687.

Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zwefel BS, Shaffer A, Talley JJ, Masferrer JL, Serbert K, Isakson PC (1998) Pharmacological analysis of cyclo-oxygenase-1 in inflammation. Proc Natl Acad Sci USA 95: 13313–13318.

Tozkoparan B, Gokhan N, Aktay G, Yesilada E, Ertan M (2000) 6-Benzylidenethiazolo [3,2-*b*]-1,2,4-triazole-5(6*H*)-ones substituted with ibuprofen: synthesis, characterization and evaluation of anti-inflammatory activity. Eur J Med Chem 35: 743–750.

Warner TD, Giuliano F, Vaynovie I, Bukasa A, Mitchell JA, Vave JR (1999) Non-steroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastro-intestinal toxicity: A full *in vitro* analysis. Proc Natl Acad Sci USA 96: 7563–7568.

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