Full Paper

Non-carboxylic Analogues of Naproxen: Design, Synthesis, and Pharmacological Evaluation of some 1,3,4-Oxadiazole/ Thiadiazole and 1,2,4-Triazole Derivatives

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A series of substituted 1,3,4-oxadiazole (**2 – 7** and **14 – 19**), 1,2,4-triazole (**20 – 25**), and 1,3,4-thiadiazole (**26 – 31**) derivatives of naproxen have been synthesized by cyclization of 2-(6-methoxy-2-naphthyl)propanoic acid hydrazide **1** and N^1[2-(6-methoxy-2-naphthyl) propanoyl]-N^4-alkyl/aryl-thiosemicarbazides (**8 – 13**) under various reaction conditions. All the compounds were screened for their anti-inflammatory activity by carrageenan-induced rat paw edema test method. Compounds showing high anti-inflammatory activity were also tested for their analgesic, ulcerogenic, and lipid peroxidation. Few of the synthesized compounds showed significant anti-inflammatory and analgesic activities along with reduced ulcerogenic effect and lipid peroxidation.

Keywords: Anti-inflammatory / Naproxen / 1,3,4-Oxadiazoles / Thiadiazoles / 1,2,4-Triazoles

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Introduction

The majority of non-steroidal anti-inflammatory drugs (NSAIDs) act via inhibition of cyclooxygenase thus preventing prostaglandin biosynthesis. However, this mechanism of action is also responsible for their main undesirable side effect, gastrointestinal (GI) ulceration and renal injury [1]. The increase in hospitalization and deaths due to GI-related disorders parallels the increased use of NSAIDs [2]. Naproxen was one of the leading NSAIDs used for relieving arthritic pain, but its long-term use invites GI complications ranging from stomach irritation to life-threatening GI ulceration and bleeding [3 – 5]. These clinical shortcomings comprise a major challenge confronting medicinal chemists to develop safer agents that spare COX-1 and subsequently its gastric cytoprotective role. Recently, it was discovered that COX exist in two isoforms, COX-1 and COX-2, which are regulated differently [6, 7]. COX-1 provides cytoprotection in the gastrointestinal tract whereas inducible COX-2 mediates inflammation [8, 9]. Thus the discovery of COX-2 provided the rationale for the development of drugs devoid of GI disorders while retaining clinical efficacy as anti-inflammatory agents. But the recent reports showed that selective COX-2 inhibitors (coxibs) could lead to adverse cardiovascular effects [10]. Therefore, development of novel compounds having anti-inflammatory and analgesic activity with an improved safety profile is still a necessity.

Synthetic approaches based upon NSAIDs chemical modification have been taken with the aim of improving their safety profile. The literature survey revealed that derivatization of the carboxylate function of NSAIDs also resulted in retained anti-inflammatory activity with reduced ulcerogenic potential [11 – 14]. Furthermore, it has been reported in the literature that certain compounds bearing 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus possess significant anti-inflammatory activity [15 – 18]. In view of these observations and in continuation of our research program on the synthesis of 5-membered heterocyclic compounds of aryl alkanoic acid

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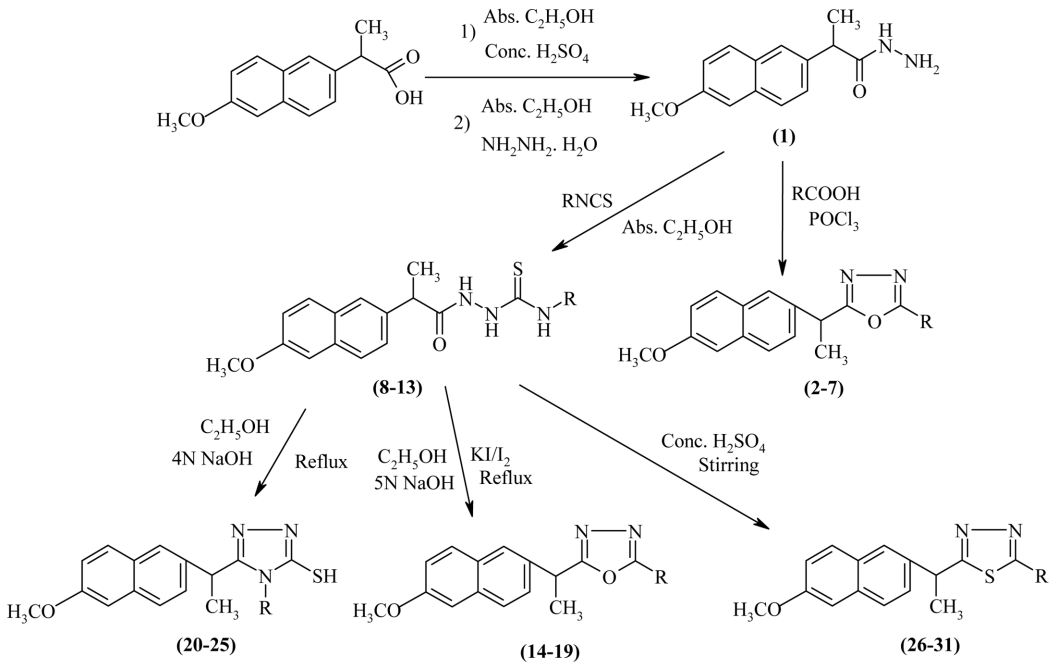
Abbreviations: carboxymethyl cellulose (CMC); gastrointestinal (GI); malondialdehyde (MDA); non-steroidal anti-inflammatory drugs (NSAIDs); serum glutamate oxaloacetate transaminase (SGOT); serum glutamate pyruvate transaminase (SGPT)



Scheme 1. Synthetic pathways to 1,3,4-oxadiazole (**2–7** and **14-19**), 1,2,4-triazole (**20–25**) and 1,3,4-thiadiazole (**26–31**) derivatives of naproxen.

derivatives [19–21], we report herein the synthesis of some newer, more potent analogues of 2-(6-methoxy-2-naphthyl)propanoic acid (naproxen) by cyclizing the carboxylic group into 1,3,4-oxadiazole, 1,2,4-triazole, and 1,3,4-thiadiazole nuclei, which have been found to possess an interesting profile of anti-inflammatory and analgesic activity with significant reduction in their ulcerogenic effect.

Results and discussion

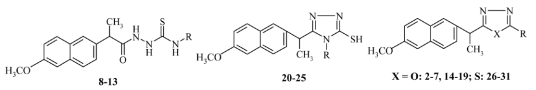
Chemistry

2-(6-Methoxy-2-naphthyl)propanoic acid hydrazide **1** was prepared by esterfication of 2-(6-methoxy-2-naphthyl)propanoic acid followed by treatment with hydrazine hydrate in absolute ethanol. Treatment of the hydrazide **1** with appropriate aromatic acids in phosphorous oxychloride afforded 5-[1-(6-methoxy-2-naphthyl)ethyl]-2-substituted-1,3,4-oxadiazoles **2–7**. Furthermore, hydrazide **1** on treatment with alkyl/aryl isothiocyanates gave N^1[2-(6-methoxy-2-naphthyl)propanoyl]-N^4-alkyl/aryl-thiosemicarbazides **8–13**. These thiosemicarbazides were oxidatively cyclized to 5-[1-(6-methoxy-2-naphthyl)ethyl]-2-alkyl/aryl amino-1,3,4-oxadiazoles **14–19** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. The thiosemicarbazides **8–13** on heating with 4N NaOH in ethanol underwent smooth cyclization through dehydration to afford 4-alkyl/aryl-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazoles **20–25**. 5-[1-(6-Methoxy-2-naphthyl)ethyl]-2-alkyl/aryl amino-1,3,4-thiadiazoles **26–31** were obtained by cyclization of thiosemicarbazides **8–13** after treatment with cold concentrated sulphuric acid (Scheme 1). The purity of various synthesized compounds was checked by TLC and elemental analysis. Spectral data (^{1}H-NMR, IR, and mass) of the synthesized compounds were in full agreement with the proposed structures.

Biological studies

The anti-inflammatory activity of the synthesized compounds **2–7**, **14–29**, and **31** was evaluated by carrageenan-induced paw edema method of Winter *et al*. [23], edema being measured after 3 and 4 h of carrageenan treatment. Also, it was observed that compounds **7, 15, 22, 25**, and **28** showed anti-inflammatory activity 78.02%, 74.99%, 75.75%, 76.51%, and 74.99% after 3 h, which was found to be greater than standard drug naproxen (74.23%). When the activity of these compounds was measured after 4 h, only compound **22** showed activity equivalent to naproxen 81.81%. The rest of the compounds showed a lower activity. Since%-inhibition was found to be more after 4 h, this was made the basis of discussion. All the derivatives of naproxen tested showed

Table 1. Physical data of the synthesized compounds.



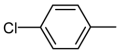
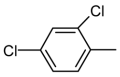
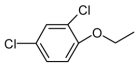
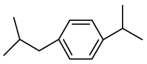
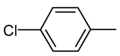
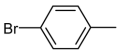
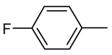
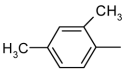
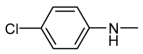
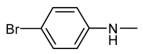
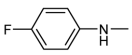
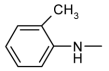
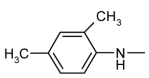
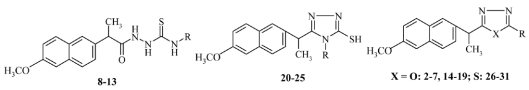
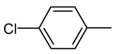
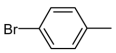
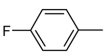
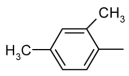
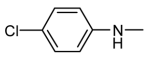
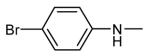
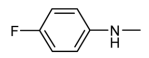
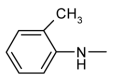
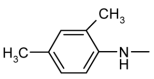
Compound	R	Yield (%)	Mp. (°C)	Mol. Formula	Mol. Wt.
2		57	186	$C_{21}H_{18}N_2O_2$	330
3		76	198	$C_{21}H_{17}ClN_2O_2$	364
4		66	>300	$C_{21}H_{17}ClN_2O_2$	364
5		59	246	$C_{21}H_{16}Cl_2N_2O_2$	399
6		68	178	$C_{22}H_{18}Cl_2N_2O_3$	429
7		66	254	$C_{27}H_{30}N_2O_2$	414
8	$CH_3CH_2CH_2CH_2$-	58	150	$C_{19}H_{25}N_3O_2S$	359
9		67	148	$C_{21}H_{20}ClN_3O_2S$	413
10		69	144	$C_{21}H_{20}BrN_3O_2S$	458
11		57	162	$C_{21}H_{20}FN_3O_2S$	397
12		54	184	$C_{22}H_{23}N_3O_2S$	393
13		61	158	$C_{23}H_{25}N_3O_2S$	407
14	$CH_3CH_2CH_2CH_2HN$-	53	128	$C_{19}H_{23}N_3O_2$	325
15		61	154	$C_{21}H_{18}ClN_3O_2$	379
16		60	>300	$C_{21}H_{18}BrN_3O_2$	424
17		55	174	$C_{21}H_{18}FN_3O_2$	363
18		51	>300	$C_{22}H_{21}N_3O_2$	359
19		54	136	$C_{23}H_{23}N_3O_2$	373

Table 1. Continued.



Compound	R	Yield (%)	Mp. (°C)	Mol. Formula	Mol. Wt.
20	$CH_3CH_2CH_2CH_2$-	76	176	$C_{19}H_{23}N_3OS$	341
21		71	124	$C_{21}H_{18}ClN_3OS$	396
22		70	210	$C_{21}H_{18}BrN_3OS$	440
23		69	144	$C_{21}H_{18}FN_3OS$	379
24		66	220	$C_{22}H_{21}N_3OS$	375
25		67	186	$C_{23}H_{23}N_3OS$	389
26	$CH_3CH_2CH_2CH_2HN$-	61	122	$C_{19}H_{23}N_3OS$	341
27		59	170	$C_{21}H_{18}ClN_3OS$	396
28		64	240	$C_{21}H_{18}BrN_3OS$	440
29		57	170	$C_{21}H_{18}FN_3OS$	379
30		61	252	$C_{22}H_{21}N_3OS$	375
31		66	216	$C_{23}H_{23}N_3OS$	389

Satisfactory analysis for CHN was obtained for all the compounds with in ±0.3% of the theoretical values.

anti-inflammatory activity ranging from 13.63 to 81.81% at an equimolar oral dose relative to 30 mg/kg naproxen after 4 h, whereas the standard drug naproxen showed 81.81% inhibition at the same oral dose (Table 2). The 1,3,4-oxadiazole derivative of naproxen **7** having 1-(4-isobutylphenyl)ethyl group at second position of the oxadiazole ring showed anti-inflammatory activity (81.05%) almost equal to the standard drug naproxen. The replacement of this group by 2,4-dichlorophenoxy methyl **6** and 4-chlorophenyl amino **15** groups resulted in a slight decrease of activity (77.19% and 77.27%, respectively). The introduction of 2-chlorophenyl **4**, 4-chlorophenyl **3**

Table 2. Anti-inflammatory activity of the synthesized compounds.

Compound	Anti-inflammatory activity % inhibition ±SEM[a)]		Compound	Anti-inflammatory activity % inhibition ±SEM[a)]	
	After 3h	After 4 h		After 3 h	After 4 h
2	26.51 ± 3.60	29.54 ± 3.05[b)]	**19**	47.72 ± 1.95	52.28 ± 2.81[b)]
3	53.78 ± 3.60	55.29 ± 3.97[b)]	**20**	18.93 ± 3.20	23.47 ± 2.47[b)]
4	62.11 ± 2.80	62.11 ± 2.80[b)]	**21**	52.26 ± 2.56	55.29 ± 2.47[b)]
5	49.24 ± 1.40	51.51 ± 0.96[b)]	**22**	75.75 ± 2.25	81.81 ± 2.04
6	73.48 ± 2.16	77.19 ± 2.37	**23**	33.33 ± 4.18	36.36 ± 4.84[b)]
7	78.02 ± 3.60	81.05 ± 3.60	**24**	17.42 ± 4.30	18.94 ± 3.79[b)]
14	45.45 ± 3.52	46.96 ± 3.45[b)]	**25**	76.51 ± 2.17	79.54 ± 1.95
15	74.99 ± 3.27	77.27 ± 2.35	**26**	71.20 ± 2.53	74.99 ± 1.94
16	11.36 ± 1.94	13.63 ± 1.66[b)]	**27**	29.54 ± 3.85	32.57 ± 3.97[b)]
17	44.69 ± 3.60	49.24 ± 3.20[b)]	**28**	74.99 ± 2.56	78.02 ± 1.82
18	52.27 ± 2.81	55.30 ± 2.73[b)]	**29**	20.45 ± 3.47	24.23 ± 3.65[b)]
Naproxen	74.23 ± 3.03	81.81 ± 2.65	**31**	38.63 ± 4.02	41.66 ± 4.30[b)]

[a)] Relative to the standard; the data was analyzed by ANOVA followed by dunnett's multiple comparision test for n = 6.
[b)] $p < 0.01$.

and 2,4-dichlorophenyl **5** groups at position two of the oxadiazole ring resulted in further decrease of activity (62.11%, 55.29%, and 51.51%, respectively). Replacement of these groups by phenyl **2** and 4-bromophenyl amino **16** groups resulted in sharp decrease of activity (29.54% and 13.63%, respectively).

1,2,4-Triazole derivatives of naproxen showed anti-inflammatory activity ranging from 18.94 to 81.81%. The maximum reduction in paw edema 81.81% (equal to naproxen) was shown by compound **22** having a 4-bromophenyl group at position four of triazole ring. When this group was replaced by the 2-methylphenyl group **24**, a sharp decrease in activity (18.94%) was observed, whereas replacement by 2,4-dimethylphenyl **25**, resulted in an only slight decrease in activity (79.54%). The other triazole derivatives showed weak anti-inflammatory activity. The cyclization of the carboxylic group of naproxen into the thiadiazole ring also showed good anti-inflammatory activity ranging from 24.23% to 78.02%. Compound **28** having a 4-bromophenyl amino group at position two of the thiadiazole ring showed 78.02% inhibition in rat paw edema. The activity was found to be slightly decreased (74.99%) when this group was replaced by a *n*-butylamino group **26**. The other compounds showed moderate to weak anti-inflammatory activity.

The compounds which showed >70% anti-inflammatory activity were further tested for their analgesic activity at the same oral dose as the one used for the anti-inflammatory activity. The analgesic activity was carried out by the tail immersion method [24] and results are presented as%-analgesia in Table 3. The analgesic activity of compounds **6, 7, 15, 22, 25, 26**, and **28** was found to be in the range from 20.8 to 86.6%. It was interesting to note that the triazole derivative of naproxen **22**, having a 4-bromophenyl group at the 4th position of triazole ring, having maximum anti-inflammatory activity also showed maximum analgesic effect 86.6%, which is more than the standard drug naproxen (73.5%). The oxadiazole derivatives **6** and **15** showed good analgesic activity (58.7% and 57.6%, respectively). The rest of the compounds showed moderate to weak analgesic activity.

The compounds, which were screened for their analgesic effect, were further tested for their acute ulcerogenicity at an equimolar oral dose relative to 90 mg/kg naproxen. The ulcerogenic activity was carried out by the method of Ciolli *et al*. [25]. Results showed that all the tested compounds exhibited reduction in the severity index (Table 3) in comparison to the standard drug naproxen (severity index 2.250 ± 0.11). The 1,3,4-thiadiazole derivative **28** having 4-bromophenyl amino group at the 2nd position of the thiadiazole ring showed minimum ulcerogenicity (severity index 0.417 ± 0.08), whereas triazole derivative **25** and thiadiazole derivative **26** showed maximum severity index (0.833 ± 0.17 and 0.833 ± 0.25, respectively). It was noted that triazole derivative **22** which showed maximum anti-inflammatory and analgesic activity also showed a very low severity index of 0.583 ± 0.08 as compared to naproxen. In general, the tested compounds showed a better GI safety profile compared to the reference drug.

All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation [26]. Lipid peroxidation was measured as nanomol of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. It has been reported in the literature that compounds showing less ulcerogenic activity also showed reduced MDA content, a

Table 3. Analgesic, ulcerogenic, and lipid peroxidation activity of selected compounds.

Compound	Analgesic activity[a]			Ulcerogenic activity (Severity index ±SEM)[d]	nmol MDA content ±SEM/100 mg tissue[d]
	Pre-treatment normal 0 h (s)	Post-treatment after 4 h (s)	% Inhibition		
6	1.05 ± 0.070	1.67 ± 0.113[c]	58.7	0.750 ± 0.17[e]	5.53 ± 0.34[e]
7	1.11 ± 0.119	1.66 ± 0.197[c]	50.2	0.583 ± 0.08[e]	5.45 ± 0.47[e]
15	1.25 ± 0.208	1.97 ± 0.193[b]	57.6	0.500 ± 0.00[e]	4.78 ± 0.21[e]
22	1.28 ± 0.149	2.40 ± 0.154[b]	86.6	0.583 ± 0.08[e]	5.43 ± 0.45[e]
25	1.54 ± 0.164	1.86 ± 0.166[c]	20.8	0.833 ± 0.17[e]	5.54 ± 0.37[e]
26	1.46 ± 0.078	2.07 ± 0.077	41.8	0.833 ± 0.25[e]	5.80 ± 0.46[e]
28	1.28 ± 0.157	1.91 ± 0.059	48.9	0.417 ± 0.08[e]	4.70 ± 0.29[e]
Naproxen	1.17 ± 0.086	2.03 ± 0.039[b]	73.5	2.250 ± 0.11	9.04 ± 0.24
Control	–	–	–	0.00	3.25 ± 0.05

[a] Relative to normal; the data was analyzed by paired student's *t*-test for n = 6.
[b] $p < 0.0001$.
[c] $p < 0.005$.
[d] Relative to standard; the data was analyzed by ANOVA followed by dunnett's multiple comparision test for n = 6.
[e] $p < 0.01$.

Table 4. Effect of compounds on serum enzymes, total protein, and total albumin.

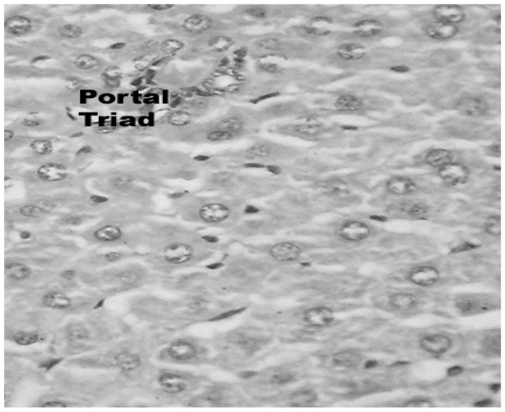
Compound	SGOT Units/ml[a]	SGPT Units/ml[a]	Alkaline Phophatase[a]	Total protein (g/dL)[a]	Total albumin (g/dL)[a]
Control	148.67 ± 1.50	27.67 ± 0.84	13.06 ± 0.25	1.80 ± 0.01	1.67 ± 0.01
7	142.17 ± 0.98[b]	25.33 ± 0.61	18.69 ± 0.16[b]	1.82 ± 0.05	1.74 ± 0.06
22	129.33 ± 0.76[b]	18.83 ± 0.60[b]	14.85 ± 0.14[b]	1.89 ± 0.12	1.74 ± 0.13
28	139.67 ± 1.65[b]	23.17 ± 0.75[b]	14.25 ± 0.14[b]	1.59 ± 0.05	1.44 ± 0.05

[a] Relative to control; the data was analyzed by ANOVA followed by dunnett's multiple comparison test, for n = 6.
[b] $p < 0.01$.

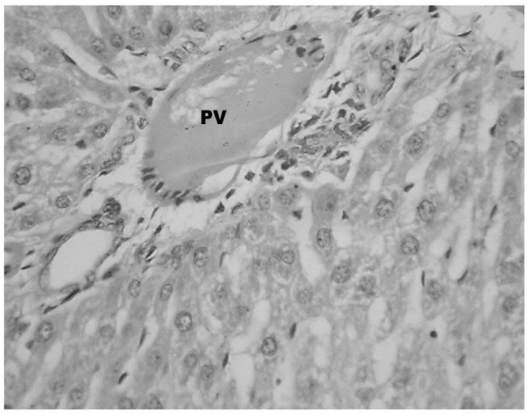
byproduct of lipid peroxidation. Naproxen (standard drug) showed maximum lipid peroxidation (9.04 ± 0.24) whereas the control group showed 3.25 ± 0.05. It was found that all cyclized derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation Table 3. Thus, these studies demonstrate that the synthesized compounds have inhibited the induction of gastric mucosal lesion.

The 1,3,4-oxadiazole, 1,2,4-triazole, and 1,3,4-thiadiazole derivatives of naproxen **7, 22**, and **28,** respectively, showing potent anti-inflammatory activity with reduced ulcerogenicity and lipid peroxidation were further studied for their hepatotoxic effect. All the compounds were studied for their effect on biochemical parameters (serum enzymes, total protein, and total albumin) [27–29] and histopathology of liver [30]. As shown in Table 4, activities of the liver enzymes serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, and total protein, total albumin almost remain the same with respect to the control values. The histopathological studies of the liver samples do not show any significant pathological changes in comparison to the control group (Fig. 1). No hepatocyte necrosis or degeneration was seen in any of the samples.

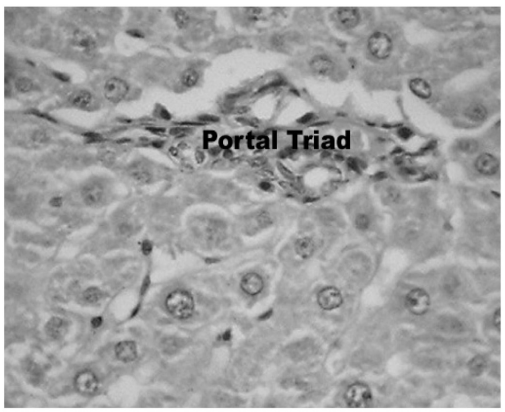
In conclusion, various oxadiazole, thiadiazole, and triazole derivatives of naproxen were prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity. It was interesting to note that seven cyclized compounds **6**, **7**, **15**, **22**, **25**, **26**, and **28** were found to have significant anti-inflammatory activity. When these compounds were subjected to analgesic activity by the tail immersion method in mice, except **25,** all compounds showed significant activity. Compound **22** was found to have a higher analgesic activity (86.6%) than the standard drug naproxen (75.5%). These compounds were also tested for ulcerogenic activity and showed a significant reduction in the severity index compared to the standard reference drug. From these studies, compound **22**, 4-(4-bromophenyl)-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4*H*)-1,2,4-triazole has emerged as the lead compound, which showed maxi-



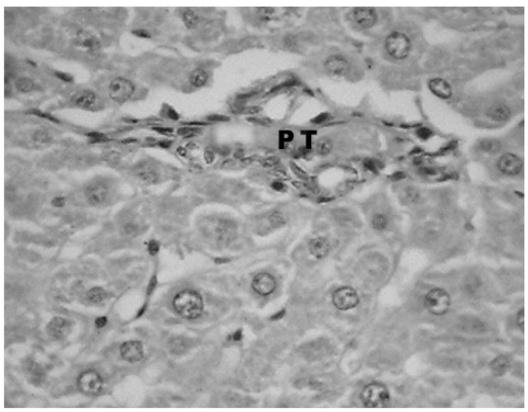
Compound **7**: Section of liver Showing Portal Triad structures (400X)



Compound **22**: Section of liver Showing Portal Triad structures (400X)



Compound **28**: Section of liver Showing Portal Triad structures (400X)



Control: Section of liver Showing Portal Triad structures (400X)

Figure 1. Histopathological studies of the liver.

mum anti-inflammatory and analgesic effects along with reduction in ulcerogenic potential and lipid peroxidation Thus the series provided new opportunities for possible modification of pharmacophoric requirements and future exploitations.

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Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet 5PC FTIR spectrometer (ν_{max} in cm^{-1}) (Nicolet, Madison, WI, USA) and ^{1}H-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) spectrometer (Bruker Bioscience, Billerica, MA, USA) using TMS as internal reference (chemical shift in δ ppm). Mass spectra were recorded at Jeol SX-102 (FAB)

spectrometer (Jeol, Tokyo, Japan). Chemicals were purchased from Merck Chemical Company,Gibbstown, NJ, USA), S. D. Fine (India) and Qualigens (India). Ethyl-2-(6-methoxy-2-naphthyl)propanoate was prepared by the procedure given in the literature [22].

Chemistry

2-(6-Methoxy-2-naphthyl)propanoic acid hydrazide ***1***

To a mixture of ethyl-2-(6-methoxy-2-naphthyl)propanoate (0.01 mol) and hydrazine hydrate (0.05 mol), absolute ethanol (50 mL) was added and it was refluxed for 24 h on a water bath. The mixture was concentrated, cooled, and poured into crushed ice. It was kept for 4 – 5 h at room temperature and the solid mass separated out was filtered, dried, and recrystallized from ethanol. Mp 94°C; Yield 61%; ^{1}H-NMR ($CDCl_3$): 1.60 (d, *J* = 7.1 Hz, 3H, CH_3); 3.66 (q, *J* = 7.1 Hz, 1H, CH); 3.92 (s, 3H, OCH_3); 6.70 (s, 2H, NH_2); 7.13 – 7.79 (m, 7H, 6-ArH and CONH).

General procedure for the synthesis of 5-[1-(6-methoxy-2-naphthyl)ethyl]-2-substituted-1,3,4-oxadiazoles ***2 – 7***

2-(6-Methoxy-2-naphthyl)propanoic acid hydrazide **1** (0.001 mol) and the appropriate aromatic acid (0.001 mol) were dissolved in phosphorus oxychloride and refluxed for 4 – 6 h. The reaction was slowly poured over crushed ice and kept overnight. Solid thus separated out was filtered, treated with dilute NaOH, washed with water, and recrystallized with ethanol.

The IR spectra of the compounds **2 – 7** showed bands at 2953 – 2929 (C-H); 1623 – 1600 (C=N) cm^{-1}.

^{1}H-NMR ($CDCl_3$) **5**: 1.67 (d, *J* = 7.0 Hz, 3H, CH_3); 3.55 (s, 3H, OCH_3); 3.92 (q, *J* = 7.0 Hz, 1H, CH); 7.13 – 8.09 (m, 9H, ArH). Mass spectra of the compound exhibited the molecular ion peak at m/z 399 [M^+], other important fragments were found at m/z 401 [M^++2], 253, 213, 185, 171.

^{1}H-NMR (DMSO-d_6) **6**: 1.48 (d, *J* = 7.0 Hz, 3H, CH_3); 3.87 – 3.93 (m, 4H, CH and OCH_3); 4.70 (s, 2H, OCH_2); 7.14 – 7.78 (m, 9H, ArH). Mass spectra of the compound exhibited the molecular ion peak at m/z 429 [M^+], other important fragments were found at m/z 431 [M^++2], 253, 213, 185, 171.

^{1}H-NMR (DMSO-d_6) **7**: 0.97 [d, *J* = 6.5 Hz, 6H, $(CH_3)_2$]; 1.43 (d, *J* = 7.0 Hz, 3H, CH_3); 1.52 (d, *J* = 7.0 Hz, 3H, CH_3); 1.84-1.92 (m, 1H, $CHCH_2$); 2.47 (d, *J* = 7.0 Hz, 2H, CH_2); 3.81-3.86 (m, 4H, CH & OCH_3); 3.94 (q, *J* = 7.0 Hz, 1H, $CHCH_3$); 6.87 – 7.92 (m, 10H, ArH). Mass spectra of the compound exhibited the molecular ion peak at m/z 414 [M^+], other important fragments were found at m/z 253, 213, 185, 171.

General procedure for the synthesis of N^1[2-(6-methoxy-2-naphthyl)propanoyl]-N^4-alkyl/aryl-thiosemicarbazides ***8 – 13***

A mixture of 2-(6-methoxy-2-naphthyl)propanoic acid hydrazide **1** (0.10 mol), alkyl/aryl isothiocyanate (0.10 mol) and ethanol (50 mL) was refluxed for 2 – 8 h on a water bath. It was then concentrated, cooled, and kept overnight in the refrigerator. The solid separated out was filtered, dried, and recrystallized with a suitable solvent. The IR spectra of the compounds **8 – 13** showed bands at 3316 – 3285 (N-H); 2955 – 2933 (C-H); 1683 – 1674 (C=O); 1108-1033 (C=S) cm^{-1}.

^{1}H-NMR ($CDCl_3$) **10**: 1.47 (d, *J* = 6.8 Hz, 3H, CH_3); 3.86 – 3.94 (m, 4H, CH and OCH_3); 7.13 – 7.79 (m, 10H, ArH); 9.60 (bs, 1H, ArNH); 9.71 (bs, 1H, CSNH); 10.17 (bs, 1H, CONH). Mass spectra of the compound exhibited the molecular ion peak at m/z 458 [M^+], other important fragments were found at m/z 460 [M^++2], 378, 245, 213, 185, 171.

^{1}H-NMR ($CDCl_3$) **11**: 1.51 (d, *J* = 6.8 Hz, 3H, CH_3); 3.82 – 3.90 (m, 4H, CH and OCH_3); 7.01 – 7.90 (m, 10H, ArH); 9.65 (bs, 1H, ArNH); 9.87 (bs, 1H, CSNH); 10.31 (bs, 1H, CONH).

^{1}H-NMR ($CDCl_3$) **12**: 1.39 (d, *J* = 6.8 Hz, 3H, CH_3); 1.94 (s, 3H, *o*-CH_3); 3.60 – 3.70 (m, 4H, CH and OCH_3); 6.79 – 7.56 (m, 10H, ArH); 8.36 (bs, 1H, ArNH); 9.03 (bs, 1H, CSNH); 9.42 (bs, 1H, CONH). Mass spectra of the compound exhibited the molecular ion peak at m/z 393 [M^+], other important fragments were found at m/z 243, 213, 185, 171.

General procedure for the synthesis of 5-[1-(6-methoxy-2-naphthyl)ethyl]-2-alkly/arylamino-1,3,4-oxadiazoles ***14 – 19***

A suspension of **8 – 13** (0.002 mol) in ethanol (50 mL) was dissolved in aqueous sodium hydroxide (5N) with cooling and stirring resulting in the formation of a clear solution. To this, iodine in potassium iodide solution (5%) was added dropwise with stirring till the color of iodine persisted at room temperature. The reaction mixture was then refluxed for 3 – 5 h on a water bath. It was then concentrated, cooled, and the solid separated out was filtered, dried, and recrystallized with ethanol. The IR spectra of the compounds **14 – 19** showed bands at 3310 – 3190 (N-H); 2954 – 2929 (C-H); 1653 – 1612 (C=N) cm^{-1}.

^{1}H-NMR ($CDCl_3$) **14**: 0.71 (t, *J* = 6.9 Hz, 3H, CH_3); 1.16 – 1.22 (m, 2H, CH_3CH_2); 1.55 – 1.62 (m, 2H, $CH_3CH_2CH_2$); 1.73 (d, *J* = 6.9 Hz, 3H, $CHCH_3$); 3.65 (t, *J* = 6.9 Hz, 2H, NCH_2); 3.91 (s, 3H, OCH_3); 4.15 (q, *J* = 6.9 Hz, 1H, CH); 7.12 – 7.70 (m, 7H, 6 ArH and NH). Mass spectra of the compound exhibited the molecular ion peak at m/z 325 [M^+], other important fragments were found at m/z 310, 253, 213, 185, 171.

^{1}H-NMR ($CDCl_3$) **15**: 1.80 (d, *J* = 7.0 Hz, 3H, CH_3); 3.90 (s, 3H, OCH_3); 4.36 (q, *J* = 7.0 Hz, 1H, CH); 7.02 – 7.73 (m, 10H, ArH); 9.88 (bs, 1H, NH). ^{1}H-NMR ($CDCl_3$) **17**: 1.80 (d, *J* = 6.9 Hz, 3H, CH_3); 3.91 (s, 3H, OCH_3); 4.36 (q, *J* = 6.9 Hz, 1H, CH); 6.93 – 7.72 (m, 10H, ArH); 9.50 (bs, 1H, NH). Mass spectra of the compound exhibited the molecular ion peak at m/z 363 (M^+), other important fragments were found at m/z 364 [M^++1], 365 [M^++2], 344, 253, 213, 185, 171.

^{1}H-NMR ($CDCl_3$) **18**: 1.79 (d, *J* = 7.0 Hz, 3H, CH_3); 2.17 (s, 3H, *o*-CH_3); 3.91 (s, 3H, OCH_3); 4.41 (q, *J* = 7.0 Hz, 1H, CH); 7.07 – 7.81 (m, 10H, ArH); 8.03 (bs, 1H, NH).

^{1}H-NMR ($CDCl_3$) **19**: 1.79 (d, *J* = 7.1 Hz, 3H, CH_3); 2.20 (s, 3H, *o*-CH_3); 2.29 (s, 3H, *p*-CH_3); 3.91 (s, 3H, OCH_3); 4.36 (q, *J* = 7.1 Hz, 1H, CH); 6.96 – 7.73 (m, 9H, ArH); 8.03 (bs, 1H, NH). Mass spectra of the compound exhibited the molecular ion peak at m/z 373 [M^+], other important fragments were found at m/z 374 [M^++1], 358, 344, 253, 213, 185, 171.

General procedure for the synthesis of 4-alkyl/aryl-5-[1-(6-methoxy-2-naphthyl)ethyl]-3-mercapto-(4H)-1,2,4-triazoles ***20 – 25***

A suspension of **8 – 13** (0.002 mol) in ethanol (50 mL) was dissolved in aqueous sodium hydroxide (4N), resulting in the formation of a clear solution. The reaction mixture was refluxed for 4 – 6 h on a water bath, concentrated, cooled, and filtered. The pH of the filtrate was adjusted between 5 – 6 with acetic acid and kept aside for 1 – 2 h. The solid separated out was filtered, washed with water, dried, and recrystallized with ethanol. The

IR spectra of the compounds **20–25** showed bands at 2950–2918 (C-H); 2786–2715 (S-H); 1638–1604 (C=N) cm^{-1}.

^{1}H-NMR ($CDCl_3$) **20**: 0.72 (t, *J* = 6.9 Hz, 3H, CH_3); 1.16–1.25 (m, 4H, $CH_3CH_2CH_2$); 1.72 (d, *J* = 6.9 Hz, 3H, $CHCH_3$); 3.81-3.92 (m, 4H, CH and OCH_3); 4.15 (t, *J* = 6.9 Hz, 2H, NCH_2); 7.12–7.73 (m, 6H, ArH); 10.83 (bs, 1H, SH). Mass spectra of the compound exhibited the molecular ion peak at m/z 341 [M^+], other important fragments were found at m/z 342 [M^++1], 308, 285, 282, 185, 171.

^{1}H-NMR ($CDCl_3$) **21**: 1.67 (d, *J* = 7.0 Hz, 3H, CH_3); 3.81–3.92 (m, 4H, CH & OCH_3); 6.81–7.61 (m, 10H, ArH); 11.79 (bs, 1H, SH). Mass spectra of the compound exhibited the molecular ion peak at m/z 396 [M^+], other important fragments were found at m/z 398 [M^++2], 363, 328, 302, 185, 171.

^{1}H-NMR ($CDCl_3$) **22**: 1.67 (d, *J* = 6.8 Hz, 3H, CH_3); 3.84–3.92 (m, 4H, CH & OCH_3); 6.74–7.62 (m, 10H, ArH); 11.27 (bs, 1H, SH). Mass spectra of the compound exhibited the molecular ion peak at m/z 440 [M^+], other important fragments were found at m/z 441 [M^++1], 442 [M^++2], 407, 327, 301, 185, 171.

^{1}H-NMR ($CDCl_3$) **23**: 1.67 (d, *J* = 7.0 Hz, 3H, CH_3); 3.87–3.92 (m, 4H, CH and OCH_3); 6.97–7.61 (m, 10H, ArH); 11.99 (bs, 1H, SH).

^{1}H-NMR ($CDCl_3$) **24**: 1.69 (d, *J* = 7.1 Hz, 3H, CH_3); 1.97 (s, 3H, CH_3); 3.84-3.90 (m, 4H, CH & OCH_3); 6.88–7.82 (m, 10H, ArH); 10.90 (bs, 1H, SH). Mass spectra of the compound exhibited the molecular ion peak at m/z 375 [M^+], other important fragments were found at m/z 342, 316, 302, 185, 171.

^{1}H-NMR ($CDCl_3$) **25**: 1.69 (d, *J* = 7.0 Hz, 3H, CH_3); 2.14 (s, 3H, *o*-CH_3); 2.43 (s, 3H, *p*-CH_3); 3.79–3.91 (m, 4H, CH and OCH_3); 6.79–7.68 (m, 9H, ArH); 11.62 (bs, 1H, SH). Mass spectra ofthe compound exhibited the molecular ion peak at m/z 389 [M^+], other important fragments were found at m/z 356, 330, 185, 171.

General procedure for the synthesis of 5-[1-(6-methoxy-2-naphthyl)ethyl]-2-alkyl/aryl amino-1,3,4-thiadiazoles ***26–31***

The thiosemicarbazide **8–13** (0.001 mol) was added gradually with stirring to cooled conc. sulphuric acid (10 mL) during 10 min. The mixture was further stirred for another 5 h in an ice bath. It was then poured over crushed ice with stirring. The solid separated out was filtered, washed with water, dried, and recrystallized with ethanol. The IR spectra of the compounds **26–31** showed bands at 3369–3186 (N-H); 2934–2900 (C-H); 1626–1606(C=N) cm^{-1}.

^{1}H-NMR (DMSO-d_6) **26**: 0.84 (t, *J* = 7.0 Hz, 3H, CH_3); 1.30–1.35 (m, 2H, CH_3CH_2); 1.53–1.57 (m, 2H, $CH_3CH_2CH_2$); 1.68 (d, *J* = 7.0 Hz, 3H, $CHCH_3$); 3.28 (t, *J* = 6.9 Hz, 2H, NCH_2); 3.83 (s, 3H, OCH_3); 4.59 (q, *J* = 7.0 Hz, 1H, CH); 7.19–7.85 (m, 6H, ArH); 8.22 (bs, 1H, NH).

^{1}H-NMR ($CDCl_3$) **27**: 1.82 (d, *J* = 7.0 Hz, 3H, CH_3); 3.91 (s, 3H, OCH_3); 4.53 (q, *J* = 7.0 Hz, 1H, CH); 7.13–7.71 (m, 10H, ArH); 8.27 (bs, 1H, NH). ^{1}H-NMR (DMSO-d_6) **28**: 1.72 (d, *J* = 6.5 Hz, 3H, CH_3); 3.83 (s, 3H, OCH_3); 4.63 (q, *J* = 7.0 Hz, 1H, CH); 7.16–7.68 (m, 10H, ArH); 8.21 (bs, 1H, NH). Mass spectra of the compound exhibited the molecular ion peak at m/z 440 [M^+], other important fragments were found at m/z 442 [M^++2], 362, 243, 255, 185.

^{1}H-NMR (DMSO-d_6) **31**: 1.72 (d, *J* = 7.0 Hz, 3H, CH_3); 2.16 (s, 3H, *o*-CH_3); 2.23 (s, 3H, *p*-CH_3); 3.82 (s, 3H, OCH_3); 4.50 (q, *J* = 7.0 Hz, 1H, CH); 7.05–7.87 (m, 9H, ArH); 8.21 (bs, 1H, NH).

Biological evaluation

Adult male Wistar strain rats of either sex, weighing 150–200 g were used. The animals were allowed food and water *ad libitum*. They were housed in a room at 25 ± 2°C, and 50 ± 5% relative humidity with 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. All the test compounds and the reference drugs were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced hind paw edema method of Winter *et al.* [23]. The animals were randomly allocated into groups of six animals each. One group was kept as control and received only 0.5% CMC solution. Group II was kept as standard and received naproxen (30 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 1 h after the administration of the test compounds and standard drugs. The right hind paw volume was measured before and after 3 and 4 h of carrageenan treatment by means of a plethysmometer. The percent anti-inflammatory activity was calculated according to the following formula.

$$\text{Percent anti-inflammatory activity} = (V_c - V_t/V_c) \times 100$$

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 the control group of rats.

Analgesic activity

Analgesic activity was evaluated by the tail immersion method [24]. Swiss albino mice allocated into different groups consisting of six animals in each of either sex, weighing 25–30 g were used for the experiment. Analgesic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 30 mg/kg naproxen. Test compounds and standard drugs were administered orally as suspension in CMC solution in water (0.5% w/v). The analgesic activity was assessed before and after an 4 h interval of administration of the test compounds and standard drugs. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5°C. The time (in seconds) for tail withdrawal from the water was taken as the reaction time with a cut-off time of immersion, set at 10 s for both controls as well as treated animals.

Acute ulcerogenicity

Acute ulcerogenicity was determined according to Cioli *et al.* [25]. The animals were allocated into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 90 mg/kg naproxen. Control group received only 0.5% CMC solution. Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal a 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 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 the control group was regarded as severity index of the gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa *et al.* [26]. 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 sulphate (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 a mixture of *n*-butanol and 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 in an UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using the extinction coefficient $1.56 \times 10^5\ cm^{-1}\ M^{-1}$.

Hepatotoxic studies

The study was carried out with Wistar albino rats of either sex weighing 150–200 g. The animals were divided into four groups, six rats in each. Group I was kept as control and receives only vehicle (0.5% w/v solution of carboxymethylcellulose in water), the other groups received test compounds at an equimolar oral dose relative to 30 mg/kg naproxen in 0.5% w/v solution of CMC in water once in a day for 15 days. After the treatment (15 days) blood was obtained from all the groups of rats by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of liver function

Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method [27]. The alkaline phosphatase, total protein, and total albumin were measured according to the reported procedures [28–29]. All the data are recorded in Table 4.

Histopathological studies of liver

The histopathological studies were carried out by reported method [30]. The rats were sacrificed under light-ether anesthesia 24 h after the last dosage. The livers were removed and washed with normal saline and stored in formalin solution. Sections of 5–6 microns in thickness were cut, stained with haematoxylin and eosin, and then studied under an electron microscope (Fig. 1).

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