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Original article

1,3,4-Oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid: Synthesis and preliminary evaluation of biological properties☆

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Abstract

A series of 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid were synthesized in order to obtain new compounds with potential anti-inflammatory activity, analgesic activity and lower ulcerogenic potential. All compounds were evaluated for their anti-inflammatory activity by the carrageenan induced rat paw edema test method. The compounds possessing potent anti-inflammatory activity were further tested for their analgesic, ulcerogenic and antioxidant activities. Out of all tested compounds, the compounds **3**, **7**, **17** and **20**, showed significant reduction in rat paw edema induced by carrageenan treatment. These compounds showed significant analgesic effect and at an equimolar oral doses relative to flurbiprofen were also found to be non-gastrotoxic in rats. Compound **17** was evaluated as the lead compound having more anti-inflammatory activity (81.81%) than the reference drug (79.54%), low ulcerogenic potential and protective effect on lipid peroxidation.

Keywords: 1,3,4-Oxadiazole/thiadiazoles; 1,2,4-Triazole; Anti-inflammatory; Analgesic; Ulcerogenic; Lipid peroxidation

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat the sign and symptoms of inflammation, particularly arthritic pain. NSAIDs exert their anti-inflammatory effect mainly through inhibition of cyclooxygenases (COXs), the key enzyme in prostaglandin (PG) biosynthesis from arachidonic acid (AA). There are at least two COX isoforms COX-1 and COX-2 [1,2]. Constitutive COX-1 is responsible for providing cytoprotection in gastrointestinal (GI) tract whereas inducible COX-2 mediates inflammation. Traditional NSAIDs such as aspirin, diclofenac, flurbiprofen and ibuprofen are non-selective; however, they show greater selectivity for COX-1 than COX-2 [3–5]. Therefore chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration. The incidence of clinically significant GI side effects is high (over 30%) and causes some patients to abandon NSAID therapy [6]. 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 [7]. Therefore, development of novel compounds having anti-inflammatory and analgesic activity with 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. Literature survey revealed that derivatization of the carboxylate function of NSAIDs resulted in retained anti-inflammatory activity with reduced ulcerogenic potential [8–11]. It has also been reported in literature that certain compounds bearing 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nucleus possess significant anti-inflammatory activity [12–15].

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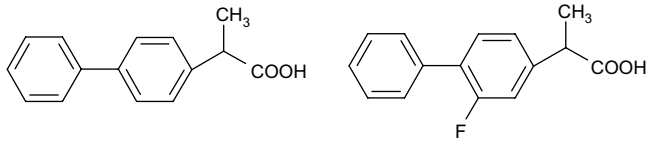
As reported [16], during the study of the pharmacological properties of a large number of substituted phenyl alkanoic acids, the most potent were found to be substituted 2-(4-biphenylyl) propionic acids and among these, flurbiprofen was discovered as possessing the most favourable therapeutic profile as an anti-inflammatory agent (Fig. 1). Furthermore, the substitution of the carboxylic acid moiety by the bioisosteric groups such as 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole has also been reported [17,18]. In view of these observations and in continuation of our research programme on the synthesis of 5-membered heterocyclic compounds of aryl alkanoic acid derivatives [19–21], we report herein the synthesis of some newer, more potent analogues of biphenyl-4-yloxy acetic acid in which the $CH{-}CH_3$ group of 2-(4-biphenylyl)propionic acid has been replaced by OCH_2 group. Thus we have designed and synthesized a series of 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole derivatives, which have been found to possess an interesting profile of anti-inflammatory and analgesic activity with significant reduction in their ulcerogenic risks in the stomach.

2. Chemistry

The hydrazide **1** was prepared by esterification of biphenyl-4-yloxy acetic acid followed by treatment with hydrazine hydrate in absolute ethanol. Various 5-[(biphenyl-4-yloxy)-methyl]-2-substituted-1,3,4-oxadiazoles **2–8** were prepared by treatment of hydrazide with appropriate aromatic acids in the presence of phosphorus oxychloride (Scheme 1). The structures of compounds were established on the basis of their elemental analysis and spectral data. The IR spectrum of compound **7** showed absorption peak at 1620 cm^{-1} due to C=N streching vibrations. Its ^1H NMR spectrum revealed a quartet and doublet at δ 3.94 and δ 1.61 for CH and CH_3 protons, respectively. Furthermore the appearance of a singlet for $(CH_3)_2$ group at δ 0.90, doublet for CH_2 group at δ 2.46 and a multiplet for CH group at δ 1.81–1.91 confirmed the presence of an isobutyl group attached to the phenyl ring. A multiplet of aromatic protons was observed at δ 6.90–7.81. The OCH_2 protons were observed as a singlet at δ 4.92. The mass spectrum of compound **7** showed molecular ion peak M^+ at *m/z* 412 corresponding to molecular formula $C_{27}H_{28}N_2O_2$. Treatment of compound **1** with various alkyl/aryl isothiocyanates in ethanol gave corresponding N^1[2-(biphenyl-4-yloxy)ethanoyl]-N^4-alkyl/aryl-thiosemicarbazides **9–14** (Table 1). The structure of thiosemicarbazide **14** was confirmed by its IR spectrum which displayed absorption peaks at 3124 cm^{-1} for NH, 1683 cm^{-1} for C=O and 1063 cm^{-1} corresponding to C=S stretching vibrations. The ^1H NMR spectrum showed a multiplet at δ 6.92–7.53 for 12 aromatic protons. The



2-(4-Biphenylyl)propionic acid **Flurbiprofen**

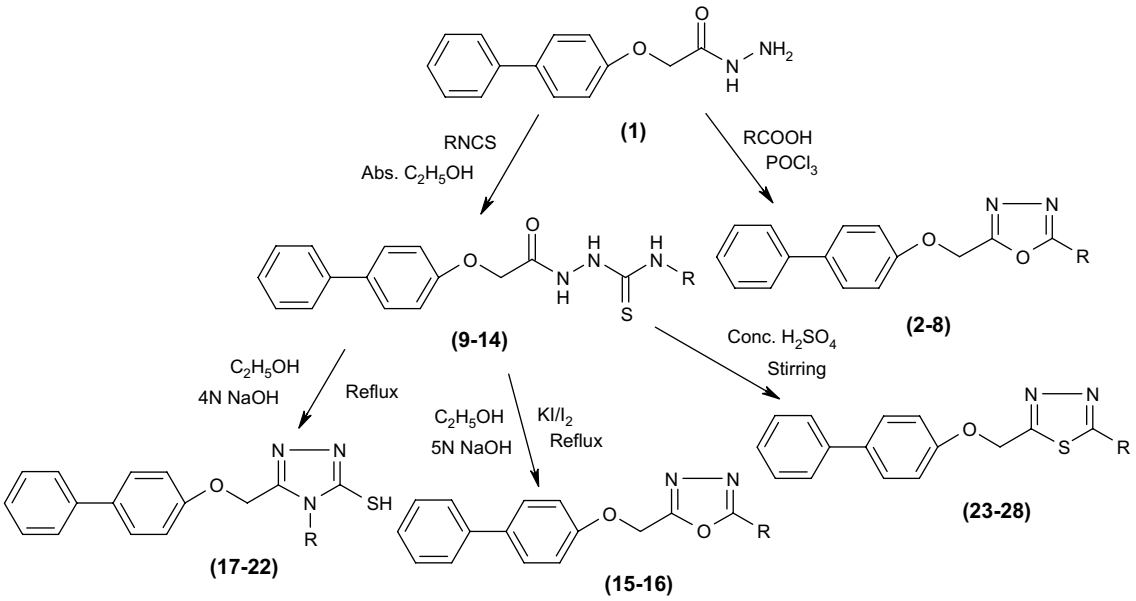
Fig. 1.

CSNH and CONH protons were observed as singlets at δ 9.50 and δ 10.00, respectively, confirming the formation of thiosemicarbazide. The mass spectrum of compound **14** showed molecular ion peak M^+ at *m/z* 405 corresponding to molecular formula $C_{23}H_{23}N_3O_2S$. The thiosemicarbazides **9** and **12** were oxidatively cyclised to 5-[(biphenyl-4-yloxy)-methyl]-2-alkyl/arylamino-1,3,4-oxadiazole **15** and **16**, by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. The IR spectra of oxadiazole **15** showed absorption peak at 1624 cm^{-1} due to C=N streching vibration. The structure was further supported by its ^1H NMR spectrum, which showed a multiplet at δ 6.88–7.61 for nine aromatic protons. The disappearance of CONH and CSNH singlet signals of thiosemicarbazide and appearance of an NH signal at δ 10.09 confirms the formation of oxadiazole ring. Mass spectra of compound **15** showed molecular ion peak M^+ at *m/z* 323 corresponding to molecular formula $C_{19}H_{21}N_3O_2$. The thiosemicarbazides **9–14** on heating with 4 N NaOH in ethanol underwent smooth cyclisation through dehydration to afford 5-[(biphenyl-4-yloxy)methyl]-4-alkyl/aryl-3-mercapto-(4*H*)-1,2,4-triazoles **17–22**. The structure of triazole **17** was confirmed by its IR spectrum, which displayed absorption peak at 1614 cm^{-1} due to C=N and at 2604 cm^{-1} corresponding to SH group. The formation of triazole ring was further supported by its ^1H NMR spectrum, which showed a triplet of NCH_2 protons at δ 4.12. A singlet of SH proton was also observed at δ 11.29. The mass spectra of compound **17** showed molecular ion peak M^+ at *m/z* 339 corresponding to molecular formula $C_{19}H_{21}N_3OS$.

5-[(Biphenyl-4-yloxy)methyl]-2-alkyl/aryl amino-1,3,4-thiadiazoles **23–28** were obtained by cyclisation of **9–14** by treating with cold concentrated sulphuric acid. The IR spectrum of compound **23** showed absorption peak at 1608 cm^{-1} due to C=N streching vibrations. In the ^1H NMR spectrum of compounds the singlets of CSNH and CONH of thiosemicarbazide had disappeared, and a multiplet was obtained in the aromatic region at δ 7.20–7.96 for nine aromatic protons. The NH proton was observed as a singlet at δ 9.97 confirming the structure of the compound. The mass spectra of compound **23** showed molecular ion peak M^+ at *m/z* 339 corresponding to molecular formula $C_{19}H_{21}N_3OS$.

3. Pharmacological results and discussion

The anti-inflammatory activity of the synthesized compounds **2–8**, **15–28** was evaluated by the carrageenan induced paw edema method of Winter et al. [23]. The compounds were tested at an equimolar oral dose relative to 10 mg/kg flurbiprofen. The percentage inhibition was calculated both after 3 and 4 h, and since it was found to be more after 4 h, this was made the basis of discussion. The tested compounds showed anti-inflammatory activity ranging from 15.90% to 81.81% (Table 2), whereas standard drug flurbiprofen showed 79.54% inhibition after 4 h. The anti-inflammatory activity of 1,3,4-oxadiazole derivatives **2–8**, **15**, **16** was in the range of 15.90% to 79.54%. The oxadiazole derivative **7** having 2-(4-isobutylphenyl)ethyl group showed



Scheme 1. Synthesis of target compounds.

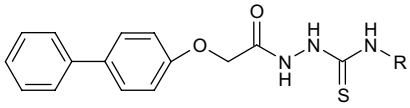
activity equivalent to the standard drug flurbiprofen (79.54%), whereas when this group was replaced by a phenyl group (**2**), activity was found to be minimal (15.90%). It was observed that oxadiazole derivatives having *p*-chlorophenyl (**3**), *o*-chlorophenyl (**4**), 2,4-dichlorophenyl (**5**) and *p*-fluorophenylamino (**16**) groups at second position showed decreasing order of anti-inflammatory activity (77.27%, 63.63%, 57.57% and 52.27%, respectively). The rest of the oxadiazole derivatives showed weak activity.

The anti-inflammatory activity of 1,2,4-triazole derivatives **17**–**22** was found between 18.18% to 81.81%. The highest activity (81.81%) was found in the triazole derivative **17** having *n*-butyl group at position 4. When this group was replaced by *p*-fluorophenyl group (**20**), the activity was slightly decreased and found equivalent to standard drug ibuprofen (79.54%). It was observed that replacement of 4-fluorophenyl group by 4-chlorophenyl group (**18**) resulted in minimum activity (18.18%). The rest of the triazole derivatives showed moderate to weak anti-inflammatory activity. The 1,3,4-thiadiazole derivatives **23**–**28** showed anti-inflammatory activity between 27.27% and 63.63%. It was observed that thiadiazole derivatives having *p*-chlorophenylamino (**24**), *p*-bromophenylamino (**25**) and 2,4-dichlorophenylamino (**28**) groups at second position showed good activity viz. 54.54%, 63.63% and 56.81%, respectively. The rest of the thiadiazole derivatives showed weak anti-inflammatory activity.

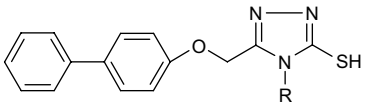
The compounds **3**, **7**, **17** and **20** which showed comparable or equal anti-inflammatory activity to that of standard reference drug, were further tested for their analgesic activity at an equimolar oral dose relative to 10 mg/kg flurbiprofen (Table 3). The compounds showed analgesic activity ranging from 16.9% to 72.8%, whereas the standard drug flurbiprofen showed 69.5% inhibition. It was noted that compound **17**, a triazole derivative showing the highest anti-inflammatory activity, also exhibited significant analgesic activity (58.2%). The highest analgesic activity (72.8%) was shown by 1,3,4-oxadiazole derivative **7**, which also has significant anti-inflammatory activity (79.54%). The remaining compounds showed reduced analgesic activity. These compounds were further screened for their acute ulcerogenic activity. The compounds were tested at an equimolar oral dose relative to 30 mg/kg flurbiprofen. The tested compounds showed significant reduction in ulcerogenic activity ranging from 0.66 ± 0.10 to 1.00 ± 0.34, whereas standard drug flurbiprofen showed high severity index of 1.66 ± 0.24. The maximum reduction in ulcerogenic activity (0.66 ± 0.10) was found in compound **17** having the *n*-butyl group at position 4 of triazole ring. The rest of the compounds also showed better GI safety profile as compared to flurbiprofen, as illustrated in Table 3. Thus the results showed that substitution of the carboxylic group by the bioisosteric groups 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole, has resulted in significant anti-inflammatory and analgesic activities along with reduced ulcerogenic potential.

It has been reported that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation [22]. 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 their effect on lipid peroxidation. Lipid peroxidation is measured as nanomoles of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Flurbiprofen exhibited maximum tissue lipid peroxidation 7.51 ± 0.68, whereas control group showed 3.25 ± 0.05. It was found that all the cyclised derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 3). Thus these studies showed that the synthesized compounds 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.

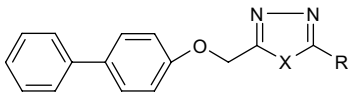
Table 1
Value of R, yields and melting points of the synthesized compounds



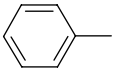
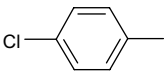
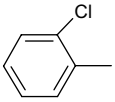
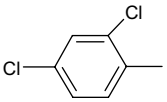
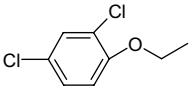
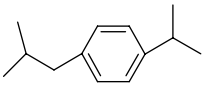
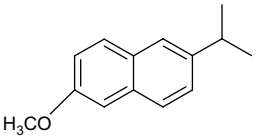
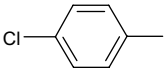
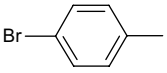
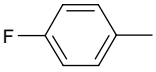
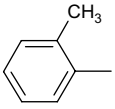
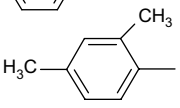
9-14



17-22



X = O: 2-8 & 15,16; S: 23-28

Compound	R	Yield (%)	M.P (°C)
2		51	134
3		50	180
4		63	102
5		47	98
6		61	58
7		58	144
8		66	122
9	$CH_3CH_2CH_2CH_2-$	67	146
10		63	182
11		69	134
12		59	174
13		55	130
14		62	104
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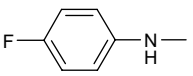
16		64	98
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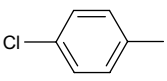
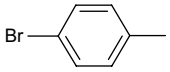
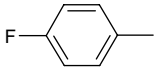
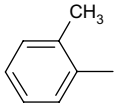
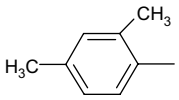
18		65	190

(*continued on next page*)

Table 1 (*continued*)

Compound	R	Yield (%)	M.P (°C)
19		71	216
20		74	188
21		68	174
22		61	170
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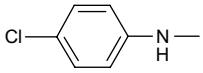
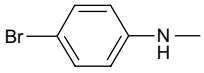
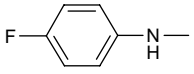
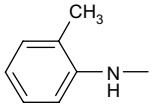
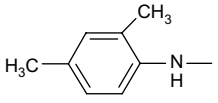
24		65	238
25		63	>300
26		60	242
27		58	178
28		43	274

Two compounds, **7** and **17**, showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation, were further studied for their hepatotoxic effect. Both compounds were studied for their effect on biochemical parameters (serum enzymes, total protein and total albumin) and histopathology of liver. As shown in Table 4 both compounds showed significant reduction in SGOT level in comparison to that of control. Compound **17** also showed significant reduction in SGPT (22.67 IU/ml) and increase in alkaline phosphatase level (16.76 g/ml) in comparison to that of control. The difference in enzyme level in biochemical studies of compounds **7** and **17** is insignificant. This is further confirmed by the histopathological studies of liver samples (Figs. 2,3), which have not shown any significant pathological changes in comparison to control group (Fig. 4). No hepatocyte necrosis or degeneration was seen in any of the samples.

4. Conclusion

We have described the preparation of 1,3,4-oxadiazole, 1,2,4-triazole and 1,3,4-thiadiazole derivatives of biphenyl-4-yloxy acetic acid. Several of these compounds have been evaluated as potential anti-inflammatory–analgesic agents with minimum ulcerogenic potential and lipid peroxidation. In conclusion, this preliminary investigation showed that the carboxylic group of biphenyl-4-yloxy acetic acid can be cyclised into 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole nuclei having significant biological activities. Among the synthesized compounds **7** and **17** possessed the most prominent and consistent activity with maximum reduction of gastrointestinal toxicity, minimum lipid peroxidation, and no hepatocyte necrosis or degeneration. Therefore this series has opened new doors for possible modifications of the pharmacophoric requirements of NSAIDs and future exploitations.

5. Experimental protocols

5.1. Chemistry

Chemicals were purchased from Merck Chemical Company, S. D. Fine (India) and Qualigens (India). 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}) and ^1H NMR spectra were recorded in

Table 2
Anti-inflammatory activity of the synthesized compounds

Compound	Paw Volume			% Inhibition ± SEM[a]		Potency
	0 h (Basal)	After 3 h	After 4 h	After 3 h	After 4 h	
2	0.35 ± 0.014	0.71 ± 0.014	0.71 ± 0.014	18.18 ± 4.23	18.18 ± 4.23**	0.22
3	0.39 ± 0.012	0.50 ± 0.014	0.49 ± 0.015	75.00 ± 2.53	77.27 ± 1.91	0.97
4	0.36 ± 0.015	0.54 ± 0.018	0.52 ± 0.017	59.09 ± 3.60	63.63 ± 3.79**	0.79
5	0.34 ± 0.009	0.54 ± 0.013	0.53 ± 0.014	56.06 ± 3.25	57.57 ± 3.45**	0.71
6	0.34 ± 0.010	0.65 ± 0.017	0.65 ± 0.016	29.54 ± 2.34	29.54 ± 2.27**	0.36
7	0.36 ± 0.017	0.46 ± 0.016	0.45 ± 0.015	77.27 ± 1.92	79.54 ± 1.91	1.00
8	0.34 ± 0.015	0.67 ± 0.018	0.67 ± 0.018	25.00 ± 1.91	25.00 ± 1.91**	0.32
15	0.31 ± 0.014	0.68 ± 0.029	0.68 ± 0.029	15.90 ± 2.56	15.90 ± 2.56**	0.20
16	0.32 ± 0.021	0.53 ± 0.015	0.53 ± 0.016	52.27 ± 2.81	52.27 ± 2.79**	0.65
17	0.32 ± 0.009	0.43 ± 0.017	0.40 ± 0.016	75.00 ± 2.73	81.81 ± 2.17	1.02
18	0.36 ± 0.016	0.73 ± 0.017	0.72 ± 0.019	15.90 ± 2.73	18.18 ± 2.25**	0.24
19	0.36 ± 0.013	0.66 ± 0.019	0.66 ± 0.017	31.81 ± 3.45	31.81 ± 3.71**	0.39
20	0.37 ± 0.011	0.48 ± 0.015	0.46 ± 0.016	75.00 ± 2.79	79.54 ± 3.19	1.00
21	0.36 ± 0.014	0.55 ± 0.015	0.54 ± 0.014	56.81 ± 1.94	59.09 ± 2.73**	0.74
22	0.35 ± 0.011	0.55 ± 0.011	0.55 ± 0.013	54.54 ± 2.62	54.54 ± 3.20**	0.67
23	0.32 ± 0.014	0.64 ± 0.021	0.64 ± 0.022	27.27 ± 2.65	27.27 ± 2.76**	0.34
24	0.34 ± 0.015	0.55 ± 0.016	0.54 ± 0.017	52.27 ± 2.17	54.54 ± 2.62**	0.67
25	0.37 ± 0.010	0.53 ± 0.014	0.53 ± 0.014	63.63 ± 1.92	63.63 ± 1.92**	0.80
26	0.37 ± 0.012	0.69 ± 0.019	0.68 ± 0.018	27.27 ± 2.73	29.54 ± 2.79**	0.37
27	0.33 ± 0.015	0.66 ± 0.022	0.64 ± 0.021	25.00 ± 1.94	29.54 ± 2.17**	0.37
28	0.35 ± 0.017	0.54 ± 0.024	0.54 ± 0.024	56.81 ± 2.55	56.81 ± 2.16**	0.71
Flurbiprofen	0.33 ± 0.011	0.44 ± 0.016	0.42 ± 0.019	75.00 ± 2.53	79.54 ± 2.25	1.00
Control	0.33 ± 0.011	0.77 ± 0.013	0.77 ± 0.016	–	–	–

[a] Relative to standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$; $^{**}p < 0.01$.

$CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) spectrometer using TMS as internal reference (Chemical shift in δ ppm). Mass spectra were recorded using Jeol SX-102 spectrometer. 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.

5.1.1. Synthesis of 2-(biphenyl-4-yloxy) acetic acid hydrazide (*1*)

Compound (**1**) was prepared by the procedure given in the literature [20].

5.1.2. Synthesis of 5-[(biphenyl-4-yloxy)methyl]-2-substituted-1,3,4-oxadiazoles (*2*–*8*)

A mixture of 2-(biphenyl-4-yloxy) acetic acid hydrazide (0.001 M) and the appropriate aromatic acid (0.001 M) in phosphorus oxychloride (10 ml) was refluxed for 4–6 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, treated with dilute NaOH, washed with water and recrystallised from ethanol.

*5.1.2.1. 5-[(Biphenyl-4-yloxy)methyl]-2-phenyl-1,3,4-oxadiazole (**2**).* IR (KBr) ν cm^{-1}: 2934 (C–H), 1612 (C=N), 1232 (C–O–C). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.27–7.61 (m, 14H, aromatic), 5.29 (s, 2H, OCH_2). MS: *m/z* 328 (M^+). Anal. Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.72; H, 4.99; N, 8.49.

*5.1.2.2. 5-[(Biphenyl-4-yloxy)methyl]-2-(4-chlorophenyl)-1,3,-4-oxadiazole (**3**).* IR (KBr) ν cm^{-1}: 2929 (C–H), 1621 (C=N), 1239 (C–O–C). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.27–8.17 (m, 13H, aromatic), 4.72 (s, 2H, OCH_2).

Table 3
Analgesic, ulcerogenic and lipid peroxidation activities of selected compounds

Compound	Analgesic Activity[a]				Ulcerogenic activity (severity index ± SEM)[b]	Nanomoles of MDA content ± SEM/100 mg tissue[b]
	Pre-treatment/normal 0 h (s) (normal)	Post-treatment/after 4 h (s)	% Inhibition	Potency		
3	1.18 ± 0.12	1.38 ± 0.143	16.9**	0.24	1.000 ± 0.34	5.88 ± 0.38
7	1.40 ± 0.150	2.42 ± 0.117	72.8***	1.04	0.750 ± 0.17*	5.28 ± 0.45**
17	1.34 ± 0.136	2.12 ± 0.144	58.2***	0.83	0.666 ± 0.10*	5.19 ± 0.22**
20	1.80 ± 0.189	2.35 ± 0.180	30.5***	0.43	0.833 ± 0.25	5.34 ± 0.37**
Flurbiprofen	1.15 ± 0.060	1.95 ± 0.097	69.5***	1.00	1.666 ± 0.24	7.51 ± 0.68
Control	–	–	–		0.00	3.25 ± 0.05

[a] Relative to normal and data were analyzed by paired student's *t*-test for $n = 6$; $^{***}p < 0.0001$, $^{**}p < 0.001$.
[b] Relative to standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$; $^{**}p < 0.01$, $^{*}p < 0.05$.

Table 4
Effect of selected compounds on serum enzymes, total proteins and total albumin

Compound	SGOT,[a] units/ml	SGPT,[a] units/ml	Alkaline phosphatase[a]	Total protein,[a] g/dl	Total albumin,[a] g/dl
Control	148.67 ± 1.50	27.67 ± 0.84	13.06 ± 0.25	1.80 ± 0.01	1.67 ± 0.01
7	141.09 ± 1.65**	29.17 ± 0.75	12.82 ± 0.16	1.92 ± 0.06	1.72 ± 0.16
17	138.00 ± 1.13**	22.67 ± 0.56**	16.76 ± 0.15**	1.82 ± 0.06	1.71 ± 0.05

[a] Relative to control and data were analyzed by ANOVA followed by Dunnett's multiple comparison test, for $n = 6$; $^{**}p < 0.01$.

MS: *m/z* 362 (M^+), 364 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{15}ClN_2O_2$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.59; H, 4.19; N, 7.68.

*5.1.2.3. 5-[(Biphenyl-4-yloxy)methyl]-2-(2-chlorophenyl)-1,3,4-oxadiazole (**4**).* IR (KBr) ν cm^{-1}: 2924 (C–H), 1610 (C=N), 1231 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 7.25–8.07 (m, 13H, aromatic), 4.65 (s, 2H, OCH_2). MS: *m/z* 362 (M^+), 364 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{15}ClN_2O_2$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.57; H, 4.23; N, 7.67.

*5.1.2.4. 5-[(Biphenyl-4-yloxy)methyl]-2-(2,4-dichlorophenyl)-1,3,4-oxadiazole (**5**).* IR (KBr) ν cm^{-1}: 2938 (C–H), 1589 (C=N), 1223 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 6.90–8.02 (m, 12H, aromatic), 4.90 (s, 2H, OCH_2). MS: *m/z* 397 (M^+), 399 ($M^+ + 2$), 401 ($M^+ + 4$). Anal. Calcd. for $C_{21}H_{14}Cl_2N_2O_2$: C, 63.49; H, 3.55; N, 7.05. Found: C, 63.57; H, 3.45; N, 7.09.

*5.1.2.5. 5-[(Biphenyl-4-yloxy)methyl]-2-(2,4-dichlorophenoxymethyl)-1,3,4-oxadiazole (**6**).* IR (KBr) ν cm^{-1}: 2922 (C–H), 1605 (C=N), 1238 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 6.86–7.81 (m, 12H, aromatic), 4.69 (s, 4H, $2OCH_2$). MS: *m/z* 427 (M^+), 429 ($M^+ + 2$), 431 ($M^+ + 4$). Anal. Calcd. for $C_{22}H_{16}Cl_2N_2O_3$: C, 61.84; H, 3.77; N, 6.56. Found: C, 61.77; H, 3.75; N, 6.59.

*5.1.2.6. 5-[(Biphenyl-4-yloxy)methyl]-2-[1-(4-isobutylphenyl)ethyl]-1,3,4-oxadiazole (**7**).* IR (KBr) ν cm^{-1}: 2956 (C–H), 1620 (C=N), 1211 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 6.90–7.81 (m, 13H, aromatic), 4.92 (s, 2H, OCH_2), 3.94 (q, $J = 7.1$ Hz, 1H, $CHCH_3$), 2.46 (d, $J = 6.5$ Hz, 2H, CH_2), 1.82–1.91 (m, 1H, CH_2CH), 1.61 (d, $J = 7.1$ Hz, 3H, CH_3), 0.90 [d, $J = 6.5$ Hz, 6H, $(CH_3)_2$]. MS: *m/z* 412 (M^+). Anal. Calcd. for $C_{27}H_{28}N_2O_2$: C, 78.61; H, 6.84; N, 6.79. Found: C, 78.57; H, 6.75; N, 6.72.

*5.1.2.7. 5-[(Biphenyl-4-yloxy)methyl]-2-[1-(6-methoxy-2-naphthyl)ethyl]-1,3,4-oxadiazole (**8**).* IR (KBr) ν cm^{-1}: 2927 (C–H), 1609 (C=N), 1243 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 7.04–7.78 (m, 15H, aromatic), 4.96 (s, 2H, OCH_2), 4.13 (q, $J = 6.6$ Hz, 3H, $CHCH_3$), 3.92 (s, 3H, OCH_3), 1.69 (d, $J = 6.6$ Hz, 3H, CH_3). MS: *m/z* 436 (M^+). Anal. Calcd. for $C_{28}H_{24}N_2O_3$: C, 77.05; H, 5.54; N, 6.42. Found: C, 77.12; H, 5.55; N, 6.39.

*5.1.3. Synthesis of N^1[2-(biphenyl-4-yloxy)ethanoyl]-N^4-alkyl/aryl-thiosemicarbazides (**9–14**)*

A mixture of 2-(biphenyl-4-yloxy) acetic acid hydrazide (0.1 M), corresponding alkyl/aryl isothiocyanate (0.1 M) and ethanol (50 ml) was refluxed for 2–9 h on a water bath. It was then concentrated, cooled and kept overnight refrigerated. The solid thus separated out was filtered, dried and recrystallised from methanol/acetone.

*5.1.3.1. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(n-butyl)-thiosemicarbazide (**9**).* IR (KBr) ν cm^{-1}: 3242 (NH), 2947 (C–H), 1673 (C=O), 1248 (C–O–C), 1083 (C=S). ^1H NMR (DMSO-d_6) δ (ppm): 10.26 (br s, 1H, CONH), 9.76 (br s, 1H, CSNH), 9.23 (br s, 1H, NH), 6.93–7.58 (m, 9H,

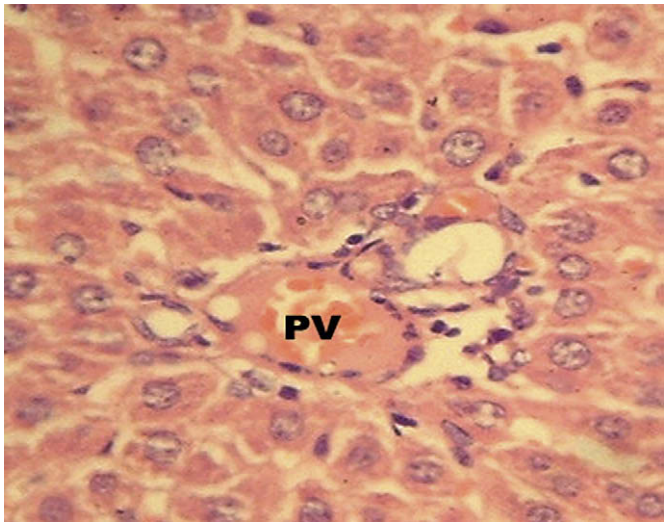


Fig. 2. Compound **7**; section of liver showing portal triad structures (400×).

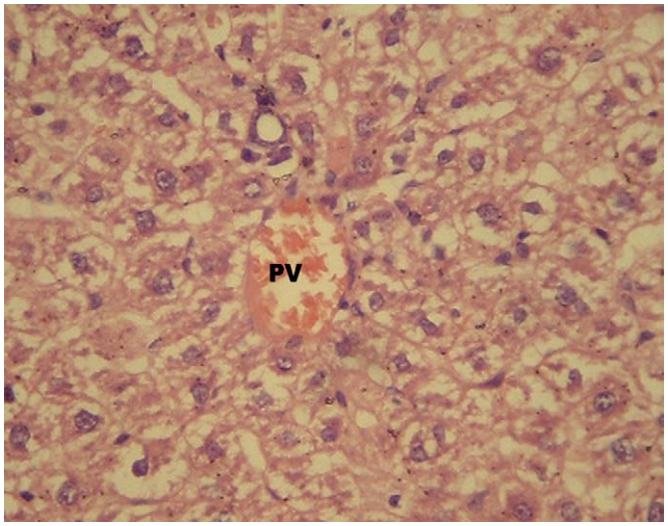


Fig. 3. Compound **17**; section of liver showing portal triad structures (400×).

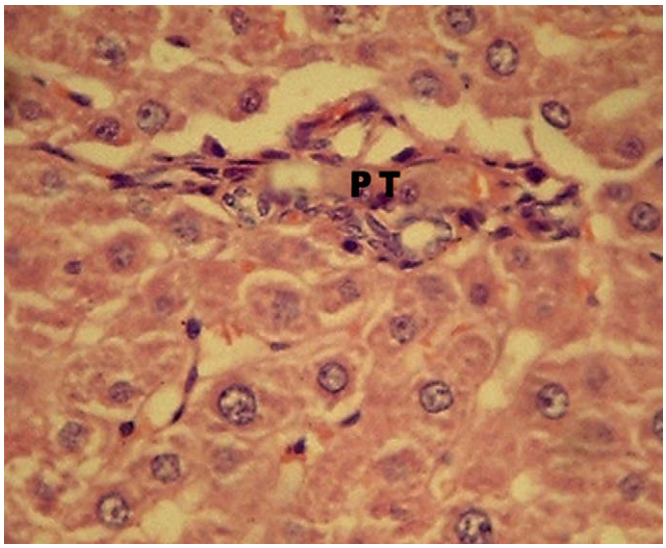


Fig. 4. Control; section of liver showing portal triad structures (400×).

aromatic), 4.58 (s, 2H, OCH_2), 3.97 (t, $J = 7.0$ Hz, 2H, NCH_2), 1.46–1.53 (m, 4H, $CH_3CH_2CH_2$), 0.98 (t, $J = 7.0$ Hz, 3H, CH_3). MS: *m/z* 357 (M^+). Anal. Calcd. for $C_{19}H_{23}N_3O_2S$: C, 63.86; H, 6.44; N, 11.76. Found: C, 63.93; H, 6.49; N, 11.82.

*5.1.3.2. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(4-chlorophenyl)-thiosemicarbazide (**10**).* IR (KBr) ν cm^{-1}: 3246 (NH), 2958 (C–H), 1683 (C=O), 1255 (C–O–C), 1099 (C=S). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.33 (br s, 1H, CONH), 9.80 (br s, 1H, CSNH), 9.67 (br s, 1H, ArNH), 7.01–7.63 (m, 13H, aromatic), 4.68 (s, 2H, OCH_2). MS: *m/z* 411 (M^+). Anal. Calcd. for $C_{21}H_{18}ClN_3O_2S$: C, 61.24; H, 4.40; N, 10.20. Found: C, 61.29; H, 4.49; N, 10.15.

*5.1.3.3. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(4-bromophenyl)-thiosemicarbazide (**11**).* IR (KBr) ν cm^{-1}: 3238 (NH), 2965 (C–H), 1686 (C=O), 1264 (C–O–C), 1085 (C=S). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.38 (br s, 1H, CONH), 9.86 (br s, 1H, CSNH), 9.73 (br s, 1H, ArNH), 7.11–7.76 (m, 13H, aromatic), 4.79 (s, 2H, OCH_2). MS: *m/z* 456 (M^+). Anal. Calcd. for $C_{21}H_{18}BrN_3O_2S$: C, 55.26; H, 3.94; N, 9.21. Found: C, 55.31; H, 3.98; N, 9.17.

*5.1.3.4. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(4-fluorophenyl)-thiosemicarbazide (**12**).* IR (KBr) ν cm^{-1}: 3258 (NH), 2969 (C–H), 1695 (C=O), 1267 (C–O–C), 1106 (C=S). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.38 (br s, 1H, CONH), 9.87 (br s, 1H, CSNH), 9.75 (br s, 1H, ArNH), 7.16–7.84 (m, 13H, aromatic), 4.68 (s, 2H, OCH_2). MS: *m/z* 395 (M^+). Anal. Calcd. for $C_{21}H_{18}FN_3O_2S$: C, 63.79; H, 4.55; N, 10.63. Found: C, 63.68; H, 4.49; N, 10.57.

*5.1.3.5. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(2-methylphenyl)-thiosemicarbazide (**13**).* IR (KBr) ν cm^{-1}: 3241 (NH), 2929 (C–H), 1670 (C=O), 1250 (C–O–C), 1100 (C=S). ^{1}H NMR ($CDCl_3$) δ (ppm): 8.82 (br s, 1H, CONH), 8.30 (br s, 2H, NHCSNHAr), 6.99–7.56 (m, 13H, ArH), 4.61 (s, 2H, OCH_2), 2.59 (s, 3H, CH_3). MS: *m/z* 391 (M^+). Anal. Calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.59; H, 5.49; N, 10.79.

*5.1.3.6. N^1[2-(Biphenyl-4-yloxy)ethanoyl]-N^4-(2,4-dimethylphenyl)-thiosemicarbazide (**14**).* IR (KBr) ν cm^{-1}: 3124 (NH), 2922 (C–H), 1683 (C=O), 1241 (C–O–C), 1063 (C=S). ^{1}H NMR ($CDCl_3$) δ (ppm): 10.00 (br s, 1H, CONH), 9.50 (br s, 1H, CSNH), 8.90 (br s, 1H, ArNH), 6.92–7.53 (m, 12H, aromatic), 4.69 (s, 2H, OCH_2), 2.95 (s, 3H, *p*-CH_3), 2.25 (s, 3H, *o*-CH_3). MS: *m/z* 405 (M^+). Anal. Calcd. for $C_{23}H_{23}N_3O_2S$: C, 68.12; H, 5.72; N, 10.26. Found: C, 68.19; H, 5.79; N, 10.19.

*5.1.4. Synthesis of 5-[(biphenyl-4-yloxy)methyl]-2-alkyl/arylamino-1,3,4-oxadiazoles (**15**,**16**)*

A suspension of corresponding N^1[2-(biphenyl-4-yloxy)ethanoyl]-N^4-alkyl/aryl-thiosemicarbazides (**9** and **12**) (0.002 M) in ethanol (50 ml) was dissolved in 5 N aq. sodium hydroxide solution (1 ml) with cooling and stirring resulting in the formation of clear solution. To this 5% iodine in potassium iodide solution was added dropwise with stirring till the colour of iodine persisted at room temperature. The reaction mixture was then refluxed for 3–5 h on water bath. It was then concentrated, cooled and the solid separated out was filtered, dried and recrystallised from ethanol.

*5.1.4.1. 5-[(Biphenyl-4-yloxy)methyl]-2-n-butylamino-1,3,4-oxadiazole (**15**).* IR (KBr) ν cm^{-1}: 3401 (NH), 2916 (C–H), 1624 (C=N), 1251 (C–O–C). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.09 (br s, 1H, NH), 6.88–7.61 (m, 9H, aromatic), 5.29 (s, 2H, OCH_2), 4.12 (t, $J = 7.0$ Hz, 2H, NCH_2), 1.50–1.56 (m, 4H, $CH_3CH_2CH_2$), 1.22 (t, $J = 7.0$ Hz, 3H, CH_3). MS: *m/z* 323 (M^+). Anal. Calcd. for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.54; N, 12.99. Found: C, 70.83; H, 6.59; N, 13.05.

*5.1.4.2. 5-[(Biphenyl-4-yloxy)methyl]-2-(4-fluorophenylamino)-1,3,4-oxadiazole (**16**).* IR (KBr) ν cm^{-1}: 3336 (NH), 2923 (C–H), 1606 (C=N), 1241 (C–O–C). ^{1}H NMR (DMSO-d_6) δ (ppm): 10.17 (br s, 1H, NH), 7.00–8.04 (m, 13H, aromatic), 5.33 (s, 2H, OCH_2). MS: *m/z* 361 (M^+). Anal. Calcd. for $C_{21}H_{16}FN_3O_2$: C, 69.80; H, 4.46; N, 11.63. Found: C, 69.87; H, 4.45; N, 11.69.

*5.1.5. Synthesis of 5-[(biphenyl-4-yloxy)methyl]-4-alkyl/aryl-3-mercapto-(4H)-1,2,4-triazoles (**17**–**22**)*

A suspension of corresponding N^1[2-(biphenyl-4-yloxy)ethanoyl]-N^4-alkyl/aryl-thiosemicarbazides (0.002 M) in ethanol (50 ml) was dissolved in 4 N aq. sodium hydroxide solution (2 ml) resulting in the formation of clear solution. The reaction mixture was refluxed for 4–6 h on water bath, concentrated, cooled and filtered. The pH of the filtrate was adjusted between 5 and 6 with acetic acid and kept aside for

1–2 h. The solid separated out was filtered, washed with water, dried and recrystallised from ethanol.

*5.1.5.1. 5-[(Biphenyl-4-yloxy)methyl]-4-n-butyl-3-mercapto-(4H)-1,2,4-triazole (**17**).* IR (KBr) ν cm^{-1}: 2949 (C–H), 2604 (SH), 1614 (C=N), 1234 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 11.29 (br s, 1H, SH), 7.04–7.56 (m, 9H, aromatic), 5.13 (s, 2H, OCH_2), 4.12 (t, $J = 7.2$ Hz, 2H, NCH_2), 1.82–1.87 (m, 2H, $CH_3CH_2CH_2$), 1.39–1.47 (m, 2H, CH_3CH_2), 0.97 (t, $J = 7.2$ Hz, 3H, CH_3). MS: *m/z* 339 (M^+). Anal. Calcd. for $C_{19}H_{21}N_3OS$: C, 67.23; H, 6.24; N, 12.38. Found: C, 67.30; H, 6.25; N, 12.49.

*5.1.5.2. 5-[(Biphenyl-4-yloxy)methyl]-4-(4-chlorophenyl)-3-mercapto-(4H)-1,2,4-triazole (**18**).* IR (KBr) ν cm^{-1}: 2912 (C–H), 2690 (SH), 1604 (C=N), 1234 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 13.61 (br s, 1H, SH), 6.76–7.85 (m, 13H, aromatic), 4.81 (s, 2H, OCH_2). MS: *m/z* 393 (M^+), 395 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{16}ClN_3OS$: C, 64.04; H, 4.09; N, 10.67. Found: C, 63.97; H, 4.01; N, 10.69.

*5.1.5.3. 5-[(Biphenyl-4-yloxy)methyl]-4-(4-bromophenyl)-3-mercapto-(4H)-1,2,4-triazole (**19**).* IR (KBr) ν cm^{-1}: 2932 (C–H), 2600 (SH), 1611 (C=N), 1244 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 13.85 (br s, 1H, SH), 6.89–7.67 (m, 13H, aromatic), 4.93 (s, 2H, OCH_2). MS: *m/z* 438 (M^+), 440 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{16}BrN_3OS$: C, 57.54; H, 3.68; N, 9.59. Found: C, 57.59; H, 3.74; N, 9.69.

*5.1.5.4. 5-[(Biphenyl-4-yloxy)methyl]-4-(4-fluorophenyl)-3-mercapto-(4H)-1,2,4-triazole (**20**).* IR (KBr) ν cm^{-1}: 2912 (C–H), 2554 (SH), 1607 (C=N), 1238 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 13.78 (br s, 1H, SH), 6.88–7.70 (m, 13H, aromatic), 4.92 (s, 2H, OCH_2). MS: *m/z* 377 (M^+), 378 ($M^+ + 1$). Anal. Calcd. for $C_{21}H_{16}FN_3OS$: C, 66.83; H, 4.27; N, 11.13. Found: C, 66.91; H, 4.21; N, 11.19.

*5.1.5.5. 5-[(Biphenyl-4-yloxy)methyl]-4-(2-methylphenyl)-3-mercapto-(4H)-1,2,4-triazole (**21**).* IR (KBr) ν cm^{-1}: 2926 (C–H), 2570 (SH), 1587 (C=N), 1239 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 13.84 (br s, 1H, SH), 6.82–7.51 (m, 13H, aromatic), 4.85 (s, 2H, OCH_2), 2.18 (s, 3H, CH_3). MS: *m/z* 373 (M^+). Anal. Calcd. for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.82; H, 5.19; N, 11.34.

*5.1.5.6. 5-[(Biphenyl-4-yloxy)methyl]-4-(2,4-dimethylphenyl)-3-mercapto-(4H)-1,2,4-triazole (**22**).* IR (KBr) ν cm^{-1}: 2932 (C–H), 2568 (SH), 1609 (C=N), 1248 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 11.33 (br s, 1H, SH), 6.84–7.51 (m, 12H, aromatic), 4.86 (s, 2H, OCH_2), 2.37 (s, 3H, *p*-CH_3), 2.16 (s, 3H, *o*-CH_3). MS: *m/z* 387 (M^+), 388 ($M^+ + 1$). Anal. Calcd. for $C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84. Found: C, 71.37; H, 5.51; N, 10.79.

*5.1.6. Synthesis of 5-[(biphenyl-4-yloxy)methyl]-2-alkyl/aryl amino-1,3,4-thiadiazoles (**23–28**)*

Corresponding N^1[2-(biphenyl-4-yloxy)ethanoyl]-N^4-alkyl/aryl-thiosemicarbazide (0.001 M) 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 ice bath. It was then poured over crushed ice with stirring. Solid separated out was filtered, washed with water, dried and recrystallised from ethanol.

*5.1.6.1. 5-[(Biphenyl-4-yloxy)methyl]-2-n-butylamino-1,3,4-thiadiazole (**23**).* IR (KBr) ν cm^{-1}: 3234 (NH), 2931 (C–H), 1608 (C=N), 1242 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 9.97 (br s, 1H, NH), 7.20–7.96 (m, 9H, aromatic), 4.97 (s, 2H, OCH_2), 3.56 (t, $J = 6.0$ Hz, 2H, NCH_2), 1.54–1.60 (m, 2H, $CH_3CH_2CH_2$), 1.31–1.38 (m, 2H, CH_3CH_2), 0.92 (t, $J = 7.1$ Hz, 3H, CH_3). MS: *m/z* 339 (M^+). Anal. Calcd. for $C_{19}H_{21}N_3OS$: C, 67.23; H, 6.24; N, 12.38. Found: C, 67.32; H, 6.19; N, 12.45.

*5.1.6.2. 5-[(Biphenyl-4-yloxy)methyl]-2-(4-chlorophenylamino)-1,3,4-thiadiazole (**24**).* IR (KBr) ν cm^{-1}: 3390 (NH), 2938 (C–H), 1638 (C=N), 1260 (C–O–C). ^1H NMR (DMSO-d_6) δ (ppm): 10.07 (br s, 1H, NH), 6.93–7.67 (m, 13H, aromatic), 5.45 (s, 2H, OCH_2). MS: *m/z* 393 (M^+), 395 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{16}ClN_3OS$: C, 64.04; H, 4.09; N, 10.67. Found: C, 63.98; H, 4.03; N, 10.61.

*5.1.6.3. 5-[(Biphenyl-4-yloxy)methyl]-2-(4-bromophenylamino)-1,3,4-thiadiazole (**25**).* IR (KBr) ν cm^{-1}: 3287 (NH), 2923 (C–H), 1624 (C=N), 1234 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 9.79 (br s, 1H, NH), 6.89–7.89 (m, 13H, aromatic), 5.03 (s, 2H, OCH_2). MS: *m/z* 438 (M^+), 440 ($M^+ + 2$). Anal. Calcd. for $C_{21}H_{16}BrN_3OS$: C, 57.54; H, 3.68; N, 9.59. Found: C, 57.29; H, 3.71; N, 9.67.

*5.1.6.4. 5-[(Biphenyl-4-yloxy)methyl]-2-(4-flourophenylamino)-1,3,4-thiadiazole (**26**).* IR (KBr) ν cm^{-1}: 3301 (NH), 2923 (C–H), 1606 (C=N), 1241 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 9.79 (br s, 1H, NH), 6.91–7.65 (m, 13H, ArH), 5.41 (s, 2H, OCH_2). MS: *m/z* 377 (M^+). Anal. Calcd. for $C_{21}H_{16}FN_3OS$: C, 66.83; H, 4.27; N, 11.13. Found: C, 66.94; H, 4.32; N, 11.06.

*5.1.6.5. 5-[(Biphenyl-4-yloxy)methyl]-2-(2-methylphenylamino)-1,3,4-thiadiazole (**27**).* IR (KBr) ν cm^{-1}: 3338 (NH), 2939 (C–H), 1619 (C=N), 1244 (C–O–C). ^1H NMR ($CDCl_3$) δ (ppm): 8.47 (br s, 1H, NH), 7.02–7.70 (m, 13H, aromatic), 5.32 (s, 2H, OCH_2), 2.18 (s, 3H, CH_3). MS: *m/z* 373 (M^+). Anal. Calcd. for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.72; H, 5.09; N, 11.30.

*5.1.6.6. 5-[(Biphenyl-4-yloxy)methyl]-2-(2,4-dimethylphenyl-amino)-1,3,4-thiadiazole (**28**).* IR (KBr) ν cm^{-1}: 3427 (NH), 2922 (C–H), 1611 (C=N), 1203 (C–O–C). ^1H NMR (DMSO-d_6) δ (ppm): 8.01 (br s, 1H, NH), 6.95–7.66 (m, 12H, aromatic), 5.40 (s, 2H, OCH_2), 2.26 (s, 3H, *p*-CH_3), 2.22 (s, 3H, *o*-CH_3). MS: *m/z* 387 (M^+). Anal. Calcd. for

$C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84. Found: C, 71.34; H, 5.50; N, 10.89.

5.2. Biological evaluation

5.2.1. Animals

Adult Wistar strain rats of either sex, weighing 150–200 g were used for anti-inflammatory, ulcerogenic and lipid peroxidation activities, whereas Swiss albino mice weighing 25–30 g were used for analgesic activity. The animals were allowed food and water *ad libitum* except during the experiments. 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. The experimental protocol was approved by the animal ethics committee of Hamdard University. All the test compounds and the reference drug were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution.

5.2.2. Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan induced hind paw edema method [23]. The animals were randomly allocated into groups of six animals each and were fasted for 24 h before the experiment with free access to water. Control group received only 0.5% carboxymethyl cellulose solution. Standard drug flurbiprofen was administered orally at a dose of 10 mg/kg. The test compounds were administered orally at an equimolar oral dose relative to 10 mg/kg flurbiprofen. Into the sub plantar region of the right hind paw of each rat, 0.1 ml of 1% carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds and standard drug. The right hind paw volume was measured before and after 3 and 4 h of carrageenan treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\text{Percent edema inhibition} = (V_c - V_t/V_c) \times 100$$

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 control group of rats.

5.2.3. Analgesic activity

Analgesic activity was evaluated by 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 10 mg/kg flurbiprofen. Test compounds and standard drugs were administered orally as suspension in carboxymethyl cellulose solution in water (0.5% w/v). The analgesic activity was assessed before and after 4 h interval of the administration of 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 control as well as treated groups of animals.

5.2.4. Acute ulcerogenicity

Acute ulcerogenicity was determined according to the method of 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 30 mg/kg flurbiprofen. Control group received only 0.5% carboxymethyl cellulose 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 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 control group was regarded as severity index of gastric mucosal damage.

5.2.5. 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 the 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 on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using extinction coefficient 1.56×10^5 cm^{-1} M^{-1}.

5.2.6. Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 g. Animals were divided in to three groups, six rats in each. Group 1 was kept as control and received only vehicle (0.5% w/v solution of carboxymethyl cellulose in water), rest of the groups received test compounds, at an equimolar oral dose relative to 10 mg/kg flurbiprofen in 0.5% w/v solution of carboxymethyl cellulose in water once in a day for 15 days. After the treatment (15 days) blood was obtained from all 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.

5.2.7. Assessment of liver function

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

5.2.8. Histopathological studies of liver

The histopathological studies were carried out by a reported method [30]. The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the livers were removed and washed with normal saline and stored in formalin solution. Sections of 5–6 μm in thickness were cut, stained with haematoxylin and eosin and then studied under a light microscope (Figs. 2–4).

5.2.9. Statistical analysis of data

Data are expressed as mean ± SEM. In anti-inflammatory, ulcerogenic and lipid peroxidation studies statistical differences between the treatments and standard were tested by one-way ANOVA followed by Dunnett's multiple comparison test. Assessment of liver function test is carried out between treatments and control group. A value of $p < 0.05$ was considered to be significant. In analgesic activity the statistical differences in treatments and standard were tested by paired student's *t*-test.

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