Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 2056–2066
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Condensed bridgehead nitrogen heterocyclic system: Synthesis and pharmacological activities of 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid

Mohd. Amir*, Harish Kumar, S.A. Javed

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India

Received 15 December 2006; received in revised form 22 September 2007; accepted 27 September 2007
Available online 6 October 2007

Abstract

Several 3,6-disubstituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles were prepared by condensation of 4-amino-5-substituted-3-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**) with various substituted aromatic acids and aryl/alkyl isothiocyanates through a one-pot reaction. These compounds were investigated for their anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation, antibacterial and antifungal activities. Some of the synthesized compounds showed potent anti-inflammatory activity along with minimal ulcerogenic effect and lipid peroxidation, compared to those of ibuprofen and flurbiprofen. Some of the tested compounds also showed moderate antimicrobial activity against tested bacterial and fungal strains.

Keywords: Triazolo-thiadiazoles; Anti-inflammatory; Ulcerogenicity; Lipid peroxidation; Hepatotoxic effect

1. Introduction

Currently available non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, flurbiprofen, fenbufen and naproxen exhibit gastric toxicity. Long-term use of these drugs has been associated with gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity [1]. The GI damage from NSAIDs is generally attributed to two factors, i.e. local irritation by the carboxylic acid moiety common to most NSAIDs (topical effect); and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis [2,3]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases (COXs) [2,4]. The chronic use of NSAIDs including ibuprofen may elicit appreciable GI toxicity [5]. Therefore synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving their safety profile.

Heterocycles bearing a symmetrical triazole or 1,3,4-thiadiazole moieties, represent an interesting class of compounds possessing a wide spectrum of biological activities such as anti-inflammatory [6–8], antiviral [9] and antimicrobial [10,11] properties. It has also been reported that derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems exert diverse pharmacological activities such as anti-inflammatory [12], antitumor [13], antifungal and antibacterial [14]. Furthermore, literature survey revealed that modification of the carboxyl function of representative NSAIDs resulted in increased anti-inflammatory activity with reduced ulcerogenic effect [15–17]. Our former studies [18,19] have shown that certain compounds bearing 1,2,4-triazole and 1,3,4-thiadiazole nuclei possess significant anti-inflammatory activity with reduced GI toxicity. It was therefore considered worthwhile to replace the carboxylic acid group of 2-(4-isobutylphenyl)propanoic acid and biphenyl-4-yloxy acetic acid by a composite system, which combines both the triazole and the thiadiazole nucleus in a ring to give a compact and planar structure. The compounds

* Corresponding author. Tel.: +91 11 26059878; fax: +91 11 26059688x5307.
E-mail address: mamir_s2003@yahoo.co.in (Mohd. Amir).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.09.025

thus synthesized have been found to possess an interesting profile of anti-inflammatory activity with significant reduction in their ulcerogenic effect. In view of the reported antimicrobial activity of triazolo-thiadiazoles, these compounds were also tested for their antibacterial and antifungal activities. Two selected compounds were also studied for their hepatotoxic effects on rat liver.

2. Chemistry

The acid hydrazides (**1a**,**b**) were prepared by esterification of 2-(4-isobutylphenyl)propanoic acid and biphenyl-4-yloxy acetic acid followed by treatment with hydrazine hydrate in absolute ethanol [18]. 4-Amino-3-substituted-5-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**) were prepared following the procedure of Reid and Heindel [20]. The acid hydrazides were allowed to react with carbon disulphide in the presence of potassium hydroxide in ethanol to afford the corresponding intermediate potassium dithiocarbazinate (**2a**,**b**). This salt underwent ring closure with an excess of 99% hydrazine hydrate to give 4-amino-3-substituted-5-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**). The resultant triazoles (**3a**,**b**) were further converted to 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles **4a**–**e**, **5a**–**f**, **6a**–**d** and **7a**–**d** through one-pot reaction by condensation with aromatic acids in the presence of phosphorus oxychloride and with aryl/alkyl isothiocyanates in the presence of DMF, respectively, as outlined in Scheme 1.

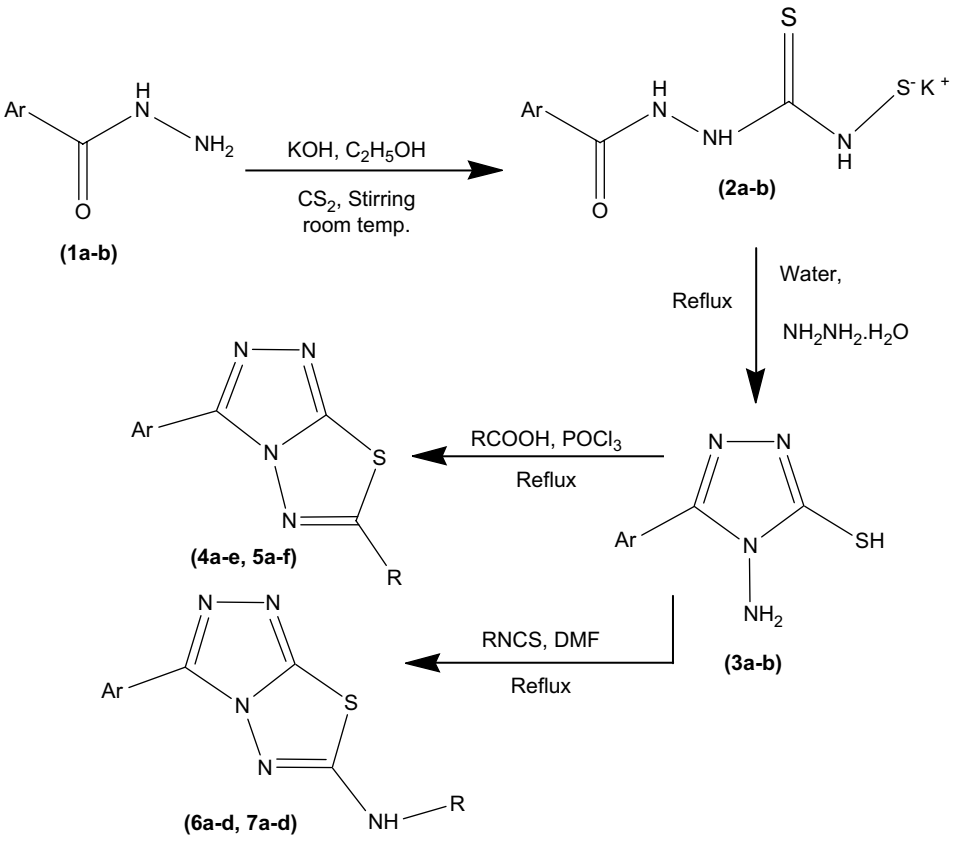
The structure of 4-amino-3-substituted-5-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**) was confirmed by ^{1}H NMR spectral data and microanalysis. The ^{1}H NMR spectra showed a downfield D_2O exchangeable singlet at δ 13.19–13.58 attributed to the SH group, while the NH_2 group appeared as a singlet at δ 5.41–5.62. The absence of signals due to NH_2 and SH protons confirmed that the triazoles were converted into triazolo-thiadiazoles (**4a**–**e**, **5a**–**f**) by reacting with COOH group of acids. Although in the ^{1}H NMR spectra of triazolo-thiadiazoles (**6a**–**d**, **7a**–**d**) the signals of NH_2 and SH protons disappeared, but a singlet of alkyl/aryl amino D_2O exchangeable proton appeared in the range of δ 8.20–13.84, confirming that triazoles were converted into triazolo-thiadiazoles (**6a**–**d**, **7a**–**d**) by reacting with alkyl/aryl isothiocyanates.

3. Results and discussion

3.1. Biological evaluation

3.1.1. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds **4a**–**e**, **5a**,**b**,**d**–**f**, **6a**–**d** and **7a**,**c**–**d** was evaluated by carrageenan-induced paw edema method of Winter et al. [21]. The compounds (**4a**–**e**, **6a**–**d**) were tested at an equimolar oral dose relative to 70 mg/kg ibuprofen and the compounds (**5a**,**b**,**d**–**f** and **7a**,**c**,**d**) were tested at an equimolar oral dose relative to 10 mg/kg flurbiprofen. The tested



Scheme 1. Synthetic pathways to 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole derivatives of ibuprofen (**4a**–**e** and **6a**–**d**) and biphenyl-4-yloxy acetic acid (**5a**–**f** and **7a**–**d**).

compounds showed anti-inflammatory activity ranging from 18.17% to 80.29%, whereas standard drugs ibuprofen and flurbiprofen showed 80.38% and 80.29% inhibition, respectively, after 4 h (Table 1). The anti-inflammatory activity of triazolo-thiadiazole derivatives of ibuprofen was in the range of 18.17–80.29%. It was observed that the triazolo-thiadiazole derivatives having a 2,4-dichlorophenyl (**4a**) and *n*-butylamino group (**6a**) at 6th position possess highest activity (80.29%). Replacement of these groups by 4-aminophenyl (**4d**) resulted in slight decrease of anti-inflammatory activity (78.78%). Further it was observed that the presence of 2,4-dichlorophenoxy methyl moiety (**4b**) at C-6 showed decrease of activity (38.63%) and replacement of this group by 2-(2,6-dichloroanilino)benzyl group resulted in sharp decrease of anti-inflammatory activity (18.17%). Compounds **4c** and **6b**,**d** showed moderate activity. The anti-inflammatory activity of triazolo-thiadiazole derivatives of biphenyl-4-yloxy acetic acid was found in the range 39.38–78.78%. The compounds having 4-chlorophenyl (**5b**), 4-aminophenyl (**5f**) and 4-fluorophenylamino (**7c**) groups at C-6 of triazolo-thiadiazole ring showed anti-inflammatory activity (78.78%, 76.51% and 78.18%, respectively) comparable to that of flurbiprofen (80.29%), the standard drug. The other compounds showed moderate activity. From the view point of structure–activity relationship (SAR), it is clear that the triazolo-thiadiazole derivatives of ibuprofen were found to be more active than biphenyl-4-yloxy acetic acid derivatives. The anti-inflammatory activity of compounds **4a** and **6a** having 2,4-dichlorophenyl and *n*-butylamino groups, respectively, was found to be the highest, being slightly less than ibuprofen, but equivalent to flurbiprofen. In general the presence of 2,4-dichlorophenyl, 4-chlorophenyl, *n*-butylamino and 4-aminophenyl groups at C-6 of triazolo-thiadiazole ring resulted in high anti-inflammatory activity.

3.1.2. Analgesic activity

The compounds that exhibited anti-inflammatory activity higher than 68% (**4a**,**d**, **5b**,**f**, **6a**,**b** and **7c**) were further tested for their analgesic activity at the same oral dose as used for the anti-inflammatory activity. All the compounds showed moderate analgesic activity in comparison to their respective standard drugs. The compounds showed analgesic activity ranging from 36.2% to 53.1% inhibition, whereas standard drugs ibuprofen and flurbiprofen showed 74.15% and 69.5% inhibition, respectively (Table 2). The analgesic activity of triazolo-thiadiazole derivatives of ibuprofen was in the range of 36.2–51.4%. The compound **6a** having *n*-butylamino group at C-6 of triazolo-thiadiazole ring showed high activity (51.4%). Replacement of this group by 2,4-dichlorophenyl (**4a**), 4-aminophenyl (**4d**) and phenyl (**6b**) groups resulted in decrease of activity (36.2%, 37.3% and 38.3%, respectively). The analgesic activity of triazolo-thiadiazole derivatives of biphenyl-4-yloxy acetic acid was found in the range of 53.1–48.4%. The compound **7c** having a 4-fluorophenylamino group showed highest analgesic activity (53.1%). Replacement of this group by 4-chlorophenyl (**5b**) and 4-aminophenyl (**5f**) groups resulted in a slight decrease of activity (48.1% and 48.4%, respectively). According to structure–activity relationship, it is clear that the triazolo-thiadiazole derivatives of biphenyl-4-yloxy acetic acid were found to be more active than ibuprofen derivatives. In general, the presence of *n*-butylamino and 4-phenylamino groups at C-6 of triazolo-thiadiazole ring resulted in good analgesic activity.

3.1.3. Acute ulcerogenesis

The compounds which were screened for analgesic activity were further tested for their acute ulcerogenic activity. Compounds **4a**,**d** and **6a**,**b** were tested at an equimolar oral dose relative to 210 mg/kg ibuprofen and compounds **5b**,**f** and **7c** were tested at an equimolar oral dose relative to 30 mg/kg flurbiprofen. The tested compounds showed low ulcerogenic activity ranging from 0.333 ± 0.10 to 1.000 ± 0.31, compared to standard drugs' ibuprofen and flurbiprofen high severity index of 2.000 ± 0.13 and 1.666 ± 0.24, respectively. The maximum reduction in ulcerogenic activity (0.333 ± 0.10) was found in the triazolo-thiadiazole derivative of ibuprofen having 2,4-dichlorophenyl group (**4a**) at C-6 of thiadiazole ring. The triazolo-thiadiazole derivative of biphenyl-4-yloxy acetic acid showing high anti-inflammatory activity (**7c**) also showed reduction in severity index (0.666 ± 0.16). The other tested compounds also exhibited better GI safety profile as compared to their standard reference drugs, as illustrated in Table 2.

3.1.4. Lipid peroxidation

It has been reported [22,23] that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation. Therefore an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation. The lipid peroxidation was measured as nmoles of MDA/100 mg of tissue. Ibuprofen and flurbiprofen exhibited high lipid peroxidation 7.79 ± 0.13 and 7.51 ± 0.68, respectively, 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 2). Thus these studies showed that 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 gastric mucosa.

3.1.5. Hepatotoxic studies

The compounds **4a** and **7c**, derivatives of 2-(4-isobutylphenyl)propanoic acid and biphenyl-4-yloxy acetic acid, respectively, showing potent anti-inflammatory activity 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 liver histopathological testing was also carried out. As shown in Table 3, activities of liver enzymes SGOT, SGPT, alkaline phosphatase, total protein and total albumin were almost identical with control values, except for compound **7c**, whose total protein and total albumin were markedly reduced. The histopathological

Table 1
Anti-inflammatory and antimicrobial activity of the compounds (**4a–e** and **5a–f**, **6a–d** and **7a–d**)

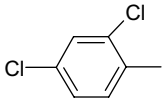
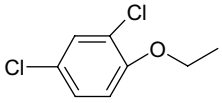
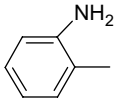
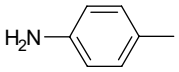
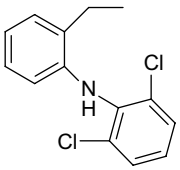
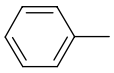
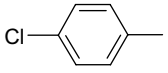
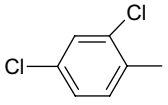
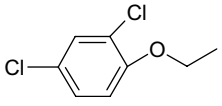
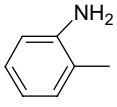
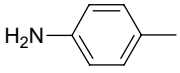
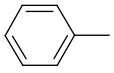
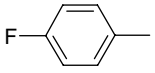
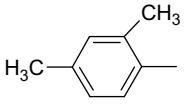
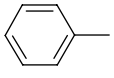
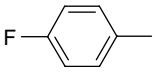
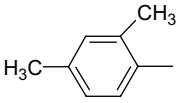
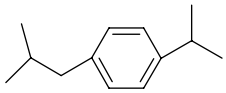
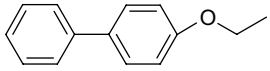
Compound	R	Ar	Anti-inflammatory activity (% inhibition ± SEM after 4 h)[e]	Antimicrobial activity (MIC μg/mL)		
				S. aureus	*E. coli*	*C. albicans*
4a		[a]	80.29 ± 1.51	200	200	200
4b		[a]	38.63 ± 3.05**	200	25	100
4c		[a]	57.57 ± 1.91**	200	100	12.5
4d		[a]	78.78 ± 1.91	200	12.5	200
4e		[a]	18.17 ± 4.23**	50	200	200
5a		[b]	65.14 ± 3.03*	25	25	[c]
5b		[b]	78.78 ± 3.03	100	25	50
5c		[b]	[d]	25	50	50
5d		[b]	50.75 ± 2.73**	25	50	50
5e		[b]	47.72 ± 2.56**	25	50	25
5f		[b]	76.51 ± 3.65	50	200	200
6a	$CH_3CH_2CH_2CH_2-$	[a]	80.29 ± 2.25	200	100	50
6b		[a]	68.17 ± 3.10*	100	25	[c]
6c		[a]	28.02 ± 2.17**	200	200	200
6d		[a]	53.02 ± 2.54**	200	50	200
7a	$CH_3CH_2CH_2CH_2-$	[b]	65.90 ± 3.05*	[c]	100	25
7b		[b]	[d]	[c]	25	200

Table 1 (*continued*)

Compound	R	Ar	Anti-inflammatory activity (% inhibition ± SEM after 4 h)[e]	Antimicrobial activity (MIC μg/mL)		
				S. aureus	*E. coli*	*C. albicans*
7c		[b]	78.18 ± 3.47	[c]	25	[c]
7d		[b]	39.38 ± 3.45**	[c]	50	100
Ibuprofen			80.38 ± 2.62	[d]	[d]	[d]
Flurbiprofen			80.29 ± 2.25	[d]	[d]	[d]
Ketoconazole			[d]	[d]	[d]	6.25
Ofloxacin			[d]	6.25	6.25	[d]
Control				[c]	[c]	[c]

*$p < 0.05$.
**$p < 0.01$.

[a] 

[b] 

[c] Did not show any activity.
[d] Not tested.
[e] Relative to their respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$.

studies of the liver samples do not show any significant pathological changes in comparison to control group (Fig. 1). No hepatocyte necrosis or degeneration was seen in any of the samples.

3.1.6. Antimicrobial activity

The compounds **4a–e**, **5a–f**, **6a–d** and **7a–d** were evaluated for their antimicrobial activity [32] against *Staphylococcus aureus* representing Gram-positive bacteria, *Escherichia coli* representing Gram-negative bacteria, and *Candida albicans* representing fungi. The results of antimicrobial effect of all the tested compounds were reported as minimal inhibitory concentrations (MICs, μg/mL), and are shown in Table 1. The results revealed that most of the newly synthesized compounds exhibited promising antibacterial activities, but they showed poor antifungal activity. Generally the test compounds showed better activity against Gram-negative bacteria (Table 1). Out of the compounds tested, **5a**,**c–e** (MIC, 25 μg/mL) exhibited moderate antibacterial activity against Gram-positive bacteria *S. aureus*, as compared to antibiotic ofloxacin (MIC, 6.25 μg/mL). It was noted that compound **4d** (MIC, 12.5 μg/mL) was 50% as active as ofloxacin (MIC, 6.25 μg/mL) against

Table 2
Analgesic, ulcerogenic and lipid peroxidation activity of selected compounds

Compound	Analgesic activity[a]			Ulcerogenic activity (severity index ± SEM)[b]	nmol MDA content ± SEM/100 mg tissue[b]
	Pre-treatment (normal 0 h (s))	Post-treatment (after 4 h (s))	% Inhibition		
4a	1.810 ± 0.186	2.467 ± 0.290	36.2	0.333 ± 0.10†	5.04 ± 0.53†
4d	0.992 ± 0.076	1.362 ± 0.139	37.3***	0.75 ± 0.25†	5.65 ± 0.36‡
5b	1.156 ± 0.067	1.706 ± 0.069	48.1*	0.833 ± 0.24‡	5.82 ± 0.50‡
5f	1.482 ± 0.153	2.200 ± 0.162	48.4*	0.417 ± 0.08 †	4.82 ± 0.25†
6a	1.217 ± 0.120	1.843 ± 0.147	51.4**	1.000 ± 0.31†	5.78 ± 0.97‡
6b	1.171 ± 0.084	1.620 ± 0.088	38.3***	0.667 ± 0.10†	5.12 ± 0.29
7c	1.860 ± 0.223	2.513 ± 0.202	53.1**	0.666 ± 0.16†	5.62 ± 0.29‡
Control	–	–	–	0.00	3.25 ± 0.05
Ibuprofen	1.361 ± 0.086	2.37 ± 0.131	74.1*	2.000 ± 0.13	7.79 ± 0.13
Flurbiprofen	1.15 ± 0.060	1.95 ± 0.097	69.5*	1.666 ± 0.24	7.51 ± 0.68

*$p < 0.0001$.
**$p < 0.001$.
***$p < 0.01$.
†$p < 0.01$.
‡$p < 0.05$.
[a] Relative to normal and data were analyzed by paired Student's *t*-test for $n = 6$.
[b] Relative to their respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$.

Table 3
Effect of compounds on serum enzymes, total proteins and total albumin

Compound	SGOT units/mL[a]	SGPT units/mL[a]	Alkaline phosphatase[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
4a	147.50 ± 0.34	28.17 ± 0.83	15.18 ± 0.13*	1.89 ± 0.07	1.80 ± 0.05**
7c	137.00 ± 0.72*	26.50 ± 0.72	17.07 ± 0.11*	0.90 ± 0.09*	0.71 ± 0.07*

*$p < 0.0001$.
**$p < 0.01$.
[a] Relative to control and data were analyzed by Student's *t*-test for $n = 6$.

E. coli. Compounds **4b**, **5a**,**b**, **6b** and **7b**,**c** exhibited only 25% (MIC, 25 μg/mL) of the activity of ofloxacin against *E. coli*. The other tested compounds were weakly active against both organisms with MIC values ranging between 50 μg/mL and 200 μg/mL. All the tested compounds showed weak antifungal activity against *C. albicans* (MIC values of 50–200 μg/mL), except for compounds **4c** (MIC, 12.5 μg/mL), **5c** and **7a** (MIC, 25 μg/mL) that showed 50%, 25% and 25% activity, respectively, as compared to the antifungal drug ketoconazole (MIC, 6.25 μg/mL).

4. Conclusions

Various triazolo-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid were synthesized and screened for anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. It was interesting to note that seven cyclised compounds **4c**,**d**, **5b**,**f**, **6a**,**b** and **7c** were found to have anti-inflammatory properties comparable to their standard reference drugs ibuprofen and flurbiprofen. When these compounds were subjected to analgesic activity by tail immersion method in mice, all compounds exhibited moderate to good activity. These compounds were also tested for ulcerogenic activity and lipid peroxidation, and showed superior GI safety profile along with reduction in lipid peroxidation as compared with ibuprofen and flurbiprofen. Compound **4d** demonstrated about half the activity of ofloxacin against *E. coli*. The other compounds showed moderate to weak antibacterial activity against *S. aureus* and *E. coli*. The synthesized compounds showed weak antifungal activity against *C. albicans*, except for compound **4c** that showed half of the activity of the antifungal drug (ketoconazole). Thus the triazolo-thiadiazole derivatives were found having dual functional properties (anti-inflammatory–analgesic and antimicrobial), and represent a promising class of compounds with an interesting pharmacological profile.

5. Experimental protocols

The melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. Elemental analysis (C, H, N, S) was performed on the CHNS Elementar (Analysen systime, GmbH) Germany Vario EL III. FTIR spectra were recorded as KBr pellets on a Jasco FT/IR 410 spectrometer and frequency was expressed in cm^{-1}. ^1H NMR spectra were recorded on a Bruker model DPX 300 NMR spectrometer. Chemical shifts (δ) are expressed in parts per million relative to tetramethylsilane (TMS); coupling constants (J) are reported in hertz, and refer to apparent peak multiplicities, and may not necessarily be true coupling constants. Mass spectra were measured on a Jeol SR-102 (FAB) mass spectrometer. Unless otherwise specified, all reactions were carried out in oven-dried glassware, and commercially available starting materials were used without further purification.

5.1. Chemistry

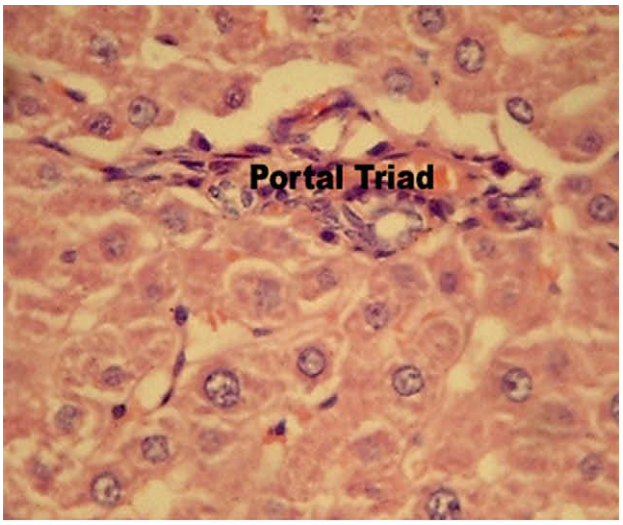
5.1.1. General method for synthesis of potassium dithiocarbazinate (*2a*,*b*)

Potassium hydroxide (0.03 M) was dissolved in absolute ethanol (50 mL). The solution was cooled in ice bath and appropriate acid hydrazide (**1a**,**b**; 0.02 M) was added with stirring. To this carbon disulphide (0.025 M) was added in small portions with constant stirring. The reaction mixture was agitated continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether (100 mL) and dried in vacuum. The potassium salt thus obtained was in quantitative yield and was used in the next step without further purification.

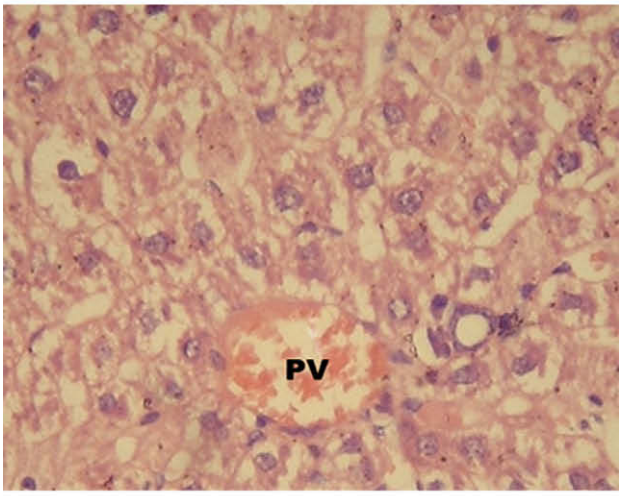
5.1.2. General method for synthesis of 4-amino-5-substituted-3-mercapto-(4H)-1,2,4-triazoles (*3a*,*b*)

A suspension of potassium dithiocarbazinate (**2a**,**b**; 0.02 M) in water (50 mL) and hydrazine hydrate (99%, 0.04 M) was refluxed for 18–20 h with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas. A homogenous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (20 mL). On acidification with acetic acid the required triazole was precipitated out. It was filtered, washed thoroughly with cold water, dried and recrystallised from ethanol. Purity of the compound was checked by TLC using silica gel-G coated plates by using toluene:ethylacetate:formic acid (T:E:F); (5:4:1) as solvent system, and observed in UV light.

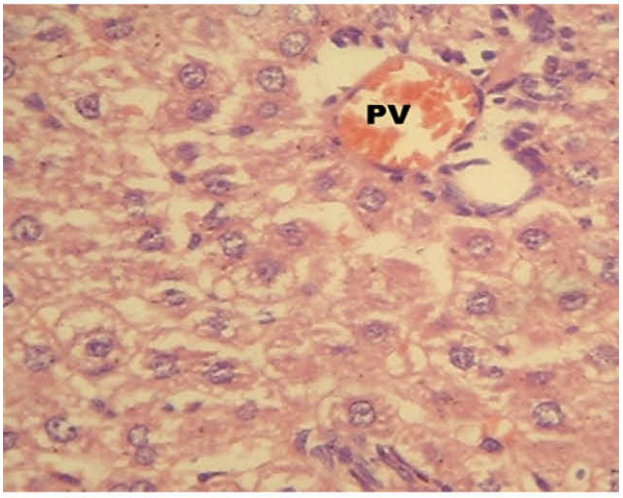
*5.1.2.1. 4-Amino-5-[1-(4-isobutylphenyl)ethyl]-3-mercapto-(4H)-1,2,4-triazole (**3a**).* M.p.: 154 °C, yield (%): 67%; IR (KBr) ν (cm^{-1}): 3315 (NH_2), 3070 (C–H aromatic), 2958 (C–H aliphatic), 2608 (SH), 1605 (C=N); ^1H NMR (DMSO-d_6) δ: 0.83 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.50 (d,



Control: Section of liver showing normal Portal Triad structures (400x)



Compound **4a:** Section of liver showing normal Portal Triad structures (400x)



Compound **7c:** Section of liver showing normal Portal Triad structures (400x)

Fig. 1. Histopathological studies of liver.

$J = 7.0$ Hz, 3H, CH_3), 1.74–1.83 (m, 1H, CH), 2.38 (d, $J = 7.0$ Hz, 2H, CH_2), 4.30 (q, $J = 7.0$ Hz, 1H, CH), 5.41 (s, 2H, NH_2), 7.10 (d, $J = 7.9$ Hz, 2H, Ar–H), 7.15 (d, $J = 7.9$ Hz, 2H, Ar–H), 13.58 (br s, 1H, SH); Mass *m/z*: 276 (M^+). Anal. Calcd. (%) for $C_{14}H_{20}N_4S$: C, 60.84; H, 7.29; N, 20.27; S, 11.60. Found: C, 60.89; H, 7.32; N, 20.23; S, 11.64.

*5.1.2.2. 4-Amino-5-[(biphenyl-4-yloxy)methyl]-3-mercapto-(4H)-1,2,4-triazole (**3b**).* M.p.: 224 °C, yield (%): 60%; IR (KBr) ν (cm^{-1}): 3290 (NH_2), 3105 (C–H aromatic), 2948 (C–H aliphatic), 2596 (SH), 1617 (C=N); ^{1}H NMR (DMSO-d_6) δ: 4.57 (s, 2H, OCH_2), 5.62 (s, 2H, NH_2), 6.83–7.58 (m, 9H, Ar–H), 13.29 (br s, 1H, SH); Mass *m/z*: 298 (M^+). Anal. Calcd. (%) for $C_{15}H_{14}N_4OS$: C, 60.38; H, 4.73; N, 18.78; S, 10.75. Found: C, 60.43; H, 4.76; N, 18.75; S, 10.71.

*5.1.3. General method for the synthesis of 6-aryl/-aryloxymethyl/substituted benzyl-3-substituted[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazoles (**4a–e**, **5a–f**)*

An equimolar mixture (0.10 M) of 4-amino-5-substituted-3-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**) and aromatic acids in phosphorus oxychloride (10 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water. The compound so obtained was dried and crystallized from ethanol.

*5.1.3.1. 6-(2,4-Dichlorophenyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**4a**).* M.p.: 96 °C, yield (%): 81%; IR (KBr) ν (cm^{-1}): 3090 (C–H aromatic), 2958 (C–H aliphatic), 1637 (C=N); ^{1}H NMR ($CDCl_3$) δ: 0.89 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.76–1.87 (m, 4H, CH and CH_3), 2.40 (d, $J = 6.5$ Hz, 2H, CH_2), 4.68 (q, $J = 7.0$ Hz, 1H, CH), 6.94–7.57 (m, 7H, Ar–H); Mass *m/z*: 431 (M^+). Anal. Calcd. (%) for $C_{21}H_{20}Cl_2N_4S$: C, 58.47; H, 4.67; N, 12.99; S, 7.43. Found: C, 58.51; H, 4.72; N, 12.95; S, 7.40.

*5.1.3.2. 6-(2,4-Dichlorophenoxymethyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4b**).* M.p.: 102 °C, yield (%): 79%; IR (KBr) ν (cm^{-1}): 3101 (C–H aromatic), 2954 (C–H aliphatic), 1610 (C=N), 1238 (C–O–C); ^{1}H NMR ($CDCl_3$) δ: 0.87 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.76–1.87 (m, 4H, CH and CH_3), 2.42 (d, $J = 6.5$ Hz, 2H, CH_2), 4.59 (q, $J = 6.5$ Hz, 1H, CH), 5.28 (s, 2H, OCH_2), 6.87–7.42 (m, 7H, Ar–H); Mass *m/z*: 461 (M^+). Anal. Calcd. (%) for $C_{22}H_{22}Cl_2N_4OS$: C, 57.27; H, 4.81; N, 12.41; S, 6.95. Found: C, 57.22; H, 4.75; N, 12.42; S, 6.92.

*5.1.3.3. 6-(2-Aminophenyl)-3-[1-(4-isobutylphenyl)ethyl-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**4c**).* M.p.: 208 °C, yield (%): 75%; IR (KBr) ν (cm^{-1}): 3346 (NH), 3098 (C–H aromatic), 2959 (C–H aliphatic), 1605 (C=N); ^{1}H NMR ($CDCl_3$) δ: 0.87 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.76–1.84 (m, 1H, CH), 1.91 (d, $J = 7.0$ Hz, 3H, CH_3), 2.43 (d, $J = 7.0$ Hz, 2H, CH_2), 4.43 (s, 2H, NH_2), 4.67 (q, $J = 7.0$ Hz, 1H, CH), 6.66–7.55 (m, 8H, Ar–H); Mass *m/z*: 377 (M^+). Anal. Calcd. (%) for

$C_{21}H_{23}N_5S$: C, 66.82; H, 6.14; N, 18.55; S, 8.49. Found: C, 66.87; H, 6.15; N, 18.59; S, 8.46.

*5.1.3.4. 6-(4-Aminophenyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**4d**).* M.p.: 140 °C, yield (%): 83%; IR (KBr) ν (cm^{-1}): 3340 (NH), 3110 (C–H aromatic), 2959 (C–H aliphatic), 1604 (C=N); ^1H NMR ($CDCl_3$) δ: 0.85 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.75–1.84 (m, 1H, CH), 1.87 (d, $J = 7.0$ Hz, 3H, CH_3), 2.41 (d, $J = 6.8$ Hz, 2H, CH_2), 4.19 (s, 2H, NH_2), 4.61 (q, $J = 7.0$ Hz, 1H, CH), 6.66 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.10 (d, $J = 6.7$ Hz, 2H, Ar–H), 7.34 (d, $J = 6.7$ Hz, 2H, Ar–H), 7.55 (d, $J = 8.0$ Hz, 2H, Ar–H); Mass *m/z*: 377 (M^+). Anal. Calcd. (%) for $C_{21}H_{23}N_5S$: C, 66.82; H, 6.14; N, 18.55; S, 8.49. Found: C, 66.86; H, 6.14; N, 18.51; S, 8.45.

*5.1.3.5. 6-[2-(2,6-Dichloroanilino)benzyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**4e**).* M.p.: 210 °C, yield (%): 78%; IR (KBr) ν (cm^{-1}): 3379 (NH), 3090 (C–H aromatic), 2959 (C–H aliphatic), 1634 (C=N); ^1H NMR (DMSO-d_6) δ: 0.84 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.56 (d, $J = 7.0$ Hz, 3H, CH_3), 1.77–1.86 (m, 1H, CH), 2.40 (d, $J = 7.0$ Hz, 2H, CH_2), 4.29 (s, 2H, CH_2), 4.54 (q, $J = 7.0$ Hz, 1H, CH), 6.90–7.85 (m, 11H, Ar–H), 9.50 (br s, 1H, NH); Mass *m/z*: 536 (M^+). Anal. Calcd. (%) for $C_{28}H_{27}Cl_2N_5S$: C, 62.68; H, 5.07; N, 13.05; S, 5.98. Found: C, 62.66; H, 5.09; N, 13.10; S, 5.94.

*5.1.3.6. 3-[(Biphenyl-4-yloxy)methyl]-6-phenyl[1,2,4]triazolo-[3,4-b][1,3,4]thiadiazole (**5a**).* M.p.: 108 °C, yield (%): 71%; IR (KBr) ν (cm^{-1}): 3061 (C–H aromatic), 2922 (C–H aliphatic), 1687 (C=N), 1272 (C–O–C); ^1H NMR ($CDCl_3$) δ: 5.40 (s, 2H, OCH_2), 7.28–8.24 (m, 14H, Ar–H); Mass *m/z*: 384 (M^+). Anal. Calcd. (%) for $C_{22}H_{16}N_4OS$: C, 68.73; H, 4.19; N, 14.57; S, 8.34. Found: C, 68.71; H, 4.17; N, 14.55; S, 8.32.

*5.1.3.7. 3-[(Biphenyl-4-yloxy)methyl]-6-(4-chlorophenyl)[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**5b**).* M.p.: 124 °C, yield (%): 69%; IR (KBr) ν (cm^{-1}): 3089 (C–H aromatic), 2932 (C–H aliphatic), 1641 (C=N), 1263 (C–O–C); ^1H NMR ($CDCl_3$) δ: 5.39 (s, 2H, OCH_2), 7.04–8.15 (m, 13H, Ar–H); Mass *m/z*: 418 (M^+). Anal. Calcd. (%) for $C_{22}H_{15}ClN_4OS$: C, 63.08; H, 3.61; N, 13.37; S, 7.65. Found: C, 63.07; H, 3.63; N, 13.39; S, 7.62.

*5.1.3.8. 3-[(Biphenyl-4-yloxy)methyl]-6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5c**).* M.p.: 172 °C, yield (%): 59%; IR (KBr) ν (cm^{-1}): 3113 (C–H aromatic), 2916 (C–H aliphatic), 1655 (C=N), 1223 (C–O–C); ^1H NMR ($CDCl_3$) δ: 5.38 (s, 2H, OCH_2), 7.21–8.19 (m, 12H, Ar–H). Anal. Calcd. (%) for $C_{22}H_{14}Cl_2N_4OS$: C, 58.29; H, 3.11; N, 12.36; S, 7.07. Found: C, 58.25; H, 3.16; N, 12.39; S, 7.05.

*5.1.3.9. 3-[(Biphenyl-4-yloxy)methyl]-6-[(2,4-dichlorophenoxy)methyl][1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**5d**).* M.p.: 300 °C, yield (%): 62%; IR (KBr) ν (cm^{-1}): 3095 (C–H aromatic), 2932 (C–H aliphatic), 1605 (C=N), 1247 (C–O–C); ^1H NMR (DMSO-d_6) δ: 5.05 (s, 4H, $2OCH_2$), 6.98–7.60 (m, 12H, Ar–H); Mass *m/z*: 483 (M^+). Anal. Calcd. (%) for $C_{23}H_{16}Cl_2N_4O_2S$: C, 57.15; H, 3.34; N, 11.59; S, 6.63. Found: C, 57.18; H, 3.30; N, 11.55; S, 6.66.

*5.1.3.10. 6-(2-Aminophenyl)-3-[(biphenyl-4-yloxy)methyl]-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**5e**).* M.p.: 248 °C, yield (%): 60%; IR (KBr) ν (cm^{-1}): 3315 (NH), 3119 (C–H aromatic), 2934 (C–H aliphatic), 1609 (C=N), 1273 (C–O–C); ^1H NMR ($CDCl_3$) δ: 4.59 (s, 2H, NH_2), 5.21 (s, 2H, OCH_2), 6.69–7.98 (m, 13H, Ar–H); Mass *m/z*: 399 (M^+). Anal. Calcd. (%) for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53; S, 8.03. Found: C, 66.11; H, 4.32; N, 17.59; S, 8.00.

*5.1.3.11. 6-(4-Aminophenyl)-3-[(biphenyl-4-yloxy)methyl]-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**5f**).* M.p.: 112 °C, yield (%): 63%; IR (KBr) ν (cm^{-1}): 3340 (NH), 3126 (C–H aromatic), 2922 (C–H aliphatic), 1601 (C=N), 1286 (C–O–C); ^1H NMR ($CDCl_3$) δ: 4.40 (s, 2H, NH_2), 5.10 (s, 2H, OCH_2), 6.69–7.98 (m, 13H, Ar–H); Mass *m/z*: 399 (M^+). Anal. Calcd. (%) for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53; S, 8.03. Found: C, 66.13; H, 4.31; N, 17.55; S, 8.01.

*5.1.4. General method for the synthesis of 6-aryl/alkylamino-3-substituted[1,2,4] triazolo[3,4-b][1,3,4]-thiadiazoles (**6a–d**, **7a–d**)*

An equimolar (0.01 M) mixture of 4-amino-5-substituted-3-mercapto-(4*H*)-1,2,4-triazoles (**3a**,**b**) and aryl/alkyl isothiocyanate in dimethylformamide (10 mL) was refluxed for 20–22 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, and washed thoroughly with cold water. The compound so obtained was dried and crystallized from ethanol/methanol.

*5.1.4.1. 6-n-Butylamino-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**6a**).* M.p.: 166 °C, yield (%): 61%; IR (KBr) ν (cm^{-1}): 3333 (NH), 3079 (C–H aromatic), 2956 (C–H aliphatic), 1611 (C=N); ^1H NMR ($CDCl_3$) δ: 0.90–0.96 (m, 9H, $(CH_3)_2$ and CH_3), 1.55–1.60 (m, 5H, CH_3 and CH_2), 1.70–1.75 (m, 2H, CH_2), 1.83–1.89 (m, 1H, CH), 2.19 (d, $J = 7.0$ Hz, 2H, Ar–CH_2), 3.90 (t, $J = 6.9$ Hz, 2H, N–CH_2), 4.39 (q, $J = 6.9$ Hz, 1H, CH), 7.11–7.69 (m, 4H, Ar–H), 9.62 (br s, 1H, NH); Mass *m/z*: 357 (M^+). Anal. Calcd. (%) for $C_{19}H_{27}N_5S$: C, 63.83; H, 7.16; N, 19.59; S, 8.97. Found: C, 63.87; H, 7.14; N, 19.61; S, 8.94.

*5.1.4.2. 3-[1-(4-Isobutylphenyl)ethyl]-6-phenylamino-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**6b**).* M.p.: 174 °C, yield (%): 65%; IR (KBr) ν (cm^{-1}): 3311 (NH), 3087 (C–H aromatic), 2958 (C–H aliphatic), 1601 (C=N); ^1H NMR (DMSO-d_6) δ: 0.85 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.59 (d, $J = 7.0$ Hz, 3H, CH_3), 1.79–1.85 (m, 1H, CH), 2.34 (d, $J = 7.0$ Hz, 3H, CH_2), 4.47 (q, $J = 7.0$ Hz, 1H, CH), 6.57–7.53 (m, 8H, Ar–H), 9.24 (br s, 1H, NH). Anal. Calcd. (%)

for $C_{21}H_{23}N_5S$: C, 66.82; H, 6.14; N, 18.55; S, 8.49. Found: C, 66.80; H, 6.11; N, 18.52; S, 8.47.

*5.1.4.3. 6-(4-Fluorophenylamino)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**6c**).* M.p.: 96 °C, yield (%): 78%; IR (KBr) ν (cm^{-1}): 3319 (NH), 3096 (C–H aromatic), 2952 (C–H aliphatic), 1598 (C=N); ^{1}H NMR ($CDCl_3$) δ: 0.87 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.65 (d, $J = 7.0$ Hz, 3H, CH_3), 1.82–1.87 (m, 1H, CH), 2.41 (d, $J = 7.0$ Hz, 2H, CH_2), 4.51 (q, $J = 7.0$ Hz, 1H, CH), 6.89–7.59 (m, 8H, Ar–H), 8.33 (br s, 1H, NH); Mass *m/z*: 395 (M^+). Anal. Calcd. (%) for $C_{21}H_{22}FN_4S$: C, 63.78; H, 5.61; N, 17.71; S, 8.11. Found: C, 63.74; H, 5.67; N, 17.77; S, 8.15.

*5.1.4.4. 6-(2,4-Dimethylphenylamino)-3-[1-(4-isobutylphenyl)-ethyl]-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**6d**).* M.p.: 140 °C, yield (%): 72%; IR (KBr) ν (cm^{-1}): 3297 (NH), 3116 (C–H aromatic), 2957 (C–H aliphatic), 1618 (C=N); ^{1}H NMR (DMSO-d_6) δ: 0.70 (d, $J = 6.5$ Hz, 6H, $(CH_3)_2$), 1.50 (d, $J = 7.0$ Hz, 3H, CH_3), 1.75–1.86 (m, 1H, CH), 2.21 (d, $J = 7.0$ Hz, 5H, CH_3 and CH_2), 2.32 (s, 3H, CH_3), 4.11 (q, $J = 7.0$ Hz, 1H, CH), 6.91–7.62 (m, 7H, Ar–H), 11.01 (br s, 1H, NH); Mass *m/z*: 405 (M^+). Anal. Calcd. (%) for $C_{23}H_{27}N_5S$: C, 68.12; H, 6.71; N, 17.27; S, 7.91. Found: C, 68.17; H, 6.68; N, 17.30; S, 7.87.

*5.1.4.5. 6-n-Butylamino-3-[(biphenyl-4-yloxy)methyl]-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**7a**).* M.p.: 240 °C, yield (%): 51%; IR (KBr) ν (cm^{-1}): 3316 (NH), 3109 (C–H aromatic), 2927 (C–H aliphatic), 1659 (C=N), 1248 (C–O–C); ^{1}H NMR ($CDCl_3$) δ: 1.21 (t, 3H, CH_3), 1.61–1.65 (m, 2H, CH_2), 2.06–2.13 (m, 2H, CH_2), 3.64 (t, 2H, N–CH_2), 4.94 (s, 2H, OCH_2), 6.95 (d, 2H, Ar–H), 7.54–7.66 (m, 7H, Ar–H), 8.20 (br s, 1H, NH); Mass *m/z*: 379 (M^+). Anal. Calcd. (%) for $C_{20}H_{21}N_5OS$: C, 63.30; H, 5.58; N, 18.46; S, 8.45. Found: C, 63.33; H, 5.55; N, 18.48; S, 8.45.

*5.1.4.6. 3-[(Biphenyl-4-yloxy)methyl]-6-phenylamino-[1,2,4]-triazolo[3,4-b][1,3,4] thiadiazole (**7b**).* M.p.: 148 °C, yield (%): 47%; IR (KBr) ν (cm^{-1}): 3420 (NH), 3104 (C–H aromatic), 2931 (C–H aliphatic), 1657 (C=N), 1251 (C–O–C); ^{1}H NMR (DMSO-d_6) δ: 5.17 (s, 2H, OCH_2), 7.12–7.63 (m, 14H, Ar–H), 13.84 (s, 1H, NH); Mass *m/z*: 399 (M^+). Anal. Calcd. (%) for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53; S, 8.03. Found: C, 66.12; H, 4.31; N, 17.56; S, 8.01.

*5.1.4.7. 3-[(Biphenyl-4-yloxy)methyl]-6-(4-fluoro phenylamino)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**7c**).* M.p.: 242 °C, yield (%): 49%; IR (KBr) ν (cm^{-1}): 3271 (NH), 3120 (C–H aromatic), 2916 (C–H aliphatic), 1611 (C=N), 1253 (C–O–C); ^{1}H NMR ($CDCl_3$) δ: 5.05 (s, 2H, OCH_2), 7.29–7.83 (m, 13H, Ar–H), 12.57 (s, 1H, NH); Mass *m/z*: 417 (M^+). Anal. Calcd. (%) for $C_{22}H_{16}FN_5OS$: C, 63.30; H, 3.86; N, 16.78; S, 7.68. Found: C, 63.27; H, 3.90; N, 16.82; S, 7.66.

*5.1.4.8. 3-[(Biphenyl-4-yloxy)methyl]-6-(2,4-dimethyl phenylamino)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (**7d**).* M.p.: 192 °C, yield: 52%; IR (KBr) ν (cm^{-1}): 3233 (NH), 3091 (C–H aromatic), 2916 (C–H aliphatic), 1605 (C=N), 1267 (C–O–C); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 5.16 (s, 2H, OCH_2), 6.72–7.47 (m, 12H, Ar–H), 12.50 (br s, 1H, NH); Mass *m/z*: 427 (M^+). Anal. Calcd. (%) for $C_{24}H_{21}N_5OS$: C, 67.43; H, 4.95; N, 16.38; S, 7.50. Found: C, 67.47; H, 4.98; N, 16.35; S, 7.54.

5.2. Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method of Winter et al [21]. The experiment was performed on Albino rats of Wistar strain of either sex, weighing 180–200 g. The animals were randomly divided into groups of six. Group I was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution. Groups II and III were kept as standard, and received ibuprofen (70 mg/kg p.o.) and flurbiprofen (10 mg/kg p.o.), respectively. 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 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 control group of rats.

5.3. Analgesic activity

Analgesic activity was evaluated by tail immersion method [24]. Swiss albino mice divided into different groups consisting of six animals each, of either sex, weighing 25–30 g, were used for the experiment. Analgesic activity was evaluated after oral administration of the test compounds (**4a**,**d** and **6a**,**b**) at an equimolar dose relative to 70 mg/kg ibuprofen, and test compounds (**5b**,**f** and **7c**) at an equimolar dose relative to 10 mg/kg flurbiprofen. 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 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 of time of immersion, set at 10 s for both control as well as treated groups of animals.

5.4. Acute ulcerogenicity

Acute ulcerogenicity was determined according to Cioli et al. [25]. The animals were divided into different groups

consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds (**4a**,**d** and **6a**,**b**) at an equimolar dose relative to 210 mg/kg ibuprofen, and test compounds (**5b**,**f** and **7c**) at an equimolar dose relative to 30 mg/kg flurbiprofen. 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 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.

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

5.6. Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 g. The animals were divided into three groups of six rats each. Group I was kept as control and received only vehicle (0.5% w/v solution of CMC in water), while group II and III received compounds **4a** and **7c**, respectively, in 0.5% w/v solution of CMC in water 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.

5.6.1. 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 reported procedures [28–30]. All the data obtained are shown in Table 3.

5.6.2. Histopathological studies of liver

Histopathological studies were carried out by reported method [31]. The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the liver was removed and washed with normal saline, and stored in formalin solution. Sections of 5–6 microns thickness were cut, stained with haematoxylin and eosin, and then studied under an electron microscope (Fig. 1).

5.7. Antibacterial and antifungal activities

Antibacterial activity of the synthesized compounds was determined *in vitro* by using cup plate method [32] against *S. aureus* (ATCC 19433) and *E. coli* (ATCC 25922) at 200 μg/mL, 100 μg/mL, 50 μg/mL and 25 μg/mL concentrations, respectively, in the nutrient agar media. Standard antibiotic ofloxacin was used as reference drug at 50 μg/mL, 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL concentrations. The test compounds which showed inhibition at 25 μg/mL concentration, were further tested at 12.5 μg/mL and 6.25 μg/mL concentrations.

Similarly, the antifungal activity of the synthesized compounds was determined *in vitro* by cup plate method against fungal strain *C. albicans* (ATCC 2091) at 200 μg/mL, 100 μg/mL, 50 μg/mL and 25 μg/mL concentrations in sabouraud dextrose medium. Ketoconazole was used as standard drug at 50 μg/mL, 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL concentrations. The test compounds which showed inhibition at 25 μg/mL concentration, were further tested at 12.5 μg/mL and 6.25 μg/mL concentrations. Solutions of required concentrations of test compounds were prepared by dissolving the compounds in DMF. The minimum inhibitory concentration (MIC) obtained for the test compounds and reference drugs are reported in Table 1. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms on the plate.

Acknowledgements

The authors are thankful to Head, Department of Pharmaceutical Chemistry for providing laboratory facilities, Central Drug Research Institute (CDRI) for spectral analysis of the compounds, and Majeedia Hospital, Hamdard University for providing necessary strains of bacteria and fungus. Authors are also thankful to Mrs. Shaukat Shah, in-charge animal house, Hamdard University for providing Wistar rats, and Dr. A. Mukherjee, MD, Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, for carrying out histopathological studies.

References

[1] M.B. Kimmey, J. Rheumatol. 19 (1992) 68–73.
[2] C.J. Smith, Y. Zhang, C.M. Koboldt, J. Muhammad, B.S. Zwefel, A. Shaffer, J.J. Talley, J.L. Masferrer, K. Serbert, P.C. Isakson, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13313–13318.

[3] C. Hawkey, L. Laine, T. Simon, A. Beaulieu, J. Maldonado-Cocco, E. Acevedo, A. Shahane, H. Quan, J. Bolognese, E. Mortensen, Arthritis. Rheum. 43 (2000) 370–377.
[4] T.D. Warner, F. Giuliano, I. Vaynovie, A. Bukasa, J.A. Mitchell, J.R. Vave, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7563–7568.
[5] F.L. Lanza, Am. J. Gastroenterol. 93 (1998) 2037–2046.
[6] D.H. Boschelli, D.T. Connor, D.A. Barnemeier, J. Med. Chem. 36 (1993) 1802–1810.
[7] P.C. Unangst, G.P. Shrum, D.T. Connor, D.R. Dyer, D.J. Schrier, J. Med. Chem. 35 (1992) 3691–3698.
[8] M. Amir, M.S.Y. Khan, M.S. Zaman, Indian J. Chem. 43B (2004) 2189–2194.
[9] S.S.P. Garoufalias, E. Tani, O. Todoulou, A.P. Valiraki, E. Filippatos, E.D. Clercq, P.N. Kourounakis, J. Pharm. Pharmacol. 50 (1998) 117–124.
[10] X.P. Hui, C.H. Zang, Q. Wang, Q. Zhang, Indian J. Chem. 41B (2002) 2176–2179.
[11] T. Tsukuda, Y. Shiratori, M.H. Watanade, K. Ontsuka, M. Hattori, N. Shirai, Shimma, Bioorg. Med. Chem. Lett. 8 (1998) 1819–1824.
[12] B. Berk, E. Aktay, E. Yesilada, M. Ertan, Pharmazie 56 (2001) 613–616.
[13] B.S. Holla, K.N. Poojary, B.S. Rao, M.K. Shivananda, Eur. J. Med. Chem. 37 (2002) 511–517.
[14] S.N. Swamy, Basappa, B.S. Priya, B. Prabhuswamy, B.H. Doreswamy, J.S. Prasad, K.S. Rangappa, Eur. J. Med. Chem. 41 (2006) 531–538.
[15] A. Kalgutar, A. Marnett, B. Crews, R. Remmel, L. Marnett, J. Med. Chem. 43 (2000) 2860–2870.
[16] M. Duflos, M. Nourrison, J. Brelet, J. Courant, G. Le Baut, N. Grimaud, J. Petit, Eur. J. Med. Chem. 36 (2001) 545–553.
[17] A. Kalgutar, B. Crews, S. Rowlinson, C. Garner, K. Siebert, L. Marnett, Science 280 (1998) 1268–1270.
[18] M. Amir, S. Kumar, Eur. J. Med. Chem. 39 (2004) 535–545.
[19] M. Amir, S. Kumar, Arch. Pharm. Chem. Life Sci. 338 (2005) 24–31.
[20] J.R. Reid, N.D. Heindel, J. Heterocycl. Chem. 13 (1976) 925–932.
[21] C.A. Winter, E.A. Risley, G.N. Nus, Proc. Soc. Exp. Biol. 111 (1962) 544–547.
[22] Y. Naito, T. Yoshikawa, N. Yoshinda, M. Kondo, Dig. Dis. Sci. 43 (1998) 30s–34s.
[23] T. Pohle, T. Brzozowski, J.C. Becker, I.R. Vander Voort, A. Markmann, S.J. Konturek, A. Moniczewski, W. Domschke, J.W. Konturek, Aliment. Pharmacol. Ther. 15 (2001) 677–687.
[24] P.A.J. Janssen, C.J.E. Niemegeers, J.G.H. Dony, Drug Res. 6 (1963) 502–507.
[25] V. Cioli, S. Putzolu, V. Rossi, S.P. Barcellona, C. Corradino, Toxicol. Appl. Pharmacol. 50 (1979) 283–289.
[26] H. Ohkawa, N. Ohishi, K. Yagi, Anal. Biochem. 95 (1979) 351–358.
[27] S. Reitman, S. Frankel, Am. J. Clin. Pathol. 28 (1957) 56.
[28] E.J. King, A.R.A. Armstrong, Can. Med. Assoc. J. 31 (1934) 376–381.
[29] J.G. Reinhold, in: M. Reiner (Ed.), Standard Methods in Clinical Chemistry, first ed. Academic Press, New York, 1953, pp. 88–90.
[30] H. Varley (Ed.), Practical Clinical Biochemistry, first ed. CBS Publishers and Distributors, New Delhi, 1988, pp. 236–238.
[31] L.G. Luna, in: Manual of Histological Staining Methods of the Armed Forces Institute of Pathology, third ed. Mc-Graw-Hill, New York, 1968, pp. 567–568.
[32] A.L. Barry, The Antimicrobial Susceptibility Test: Principle and Practices, Lea and Febiger, Philadelphia, USA, 1976, p. 180.