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Original article

Regioselective reaction: Synthesis and pharmacological study of Mannich bases containing ibuprofen moiety

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ABSTRACT

A series of 4-[(4-aryl)methylidene]amino-2-(substituted-4-ylmethyl)-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**6**) were synthesized from an arylpropionic acid namely, ibuprofen by a three-component Mannich reaction. Aminomethylation of 4-[(4-aryl)methylidene]amino-5-{1-[4-(2-methylpropyl)phenyl] ethyl}-4*H*-1,2,4-triazole-3-thiol (**5**) with formaldehyde and a secondary amine furnished this novel series of Mannich bases (**6**). Both Schiff bases (**5**) and Mannich bases (**6**) were well characterized on the basis of IR, NMR, mass spectra1 data and elemental analysis. They were screened for their anti-inflammatory, analgesic, antibacterial and antifungal activities. Some of the Mannich bases (**6**) carrying morpholino and N-methylpiperazino residues were found to be promising anti-inflammatory and analgesic agents.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are widely used in the treatment of pain and inflammation, including osteoarthritis and rheumatoid arthritis [1–3]. Prostaglandins (PGs) are well known to be the mediators of inflammation, pain and swelling. They are produced by the action of cyclooxygenase (COX) enzyme on arachidonic acid. Metabolites of the COX pathway are widely accepted as mediators of the inflammatory response. COX is known to be the principal target of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs, block the formation of PGs and have analgesic, antipyretic and anti-inflammatory activities [4]. In the early 1990s, it was discovered that the COX enzyme exists as two isoforms, one constitutive (COX-1) and the other inducible (COX-2) [5]. COX-1 is constitutively expressed and provides cytoprotection in the gastrointestinal (GI) tract while COX-2 is inducible and mediates inflammation [6–8]. The traditional NSAIDs show greater selectivity for COX-1 than COX-2 [9].

In fact, prolonged use of NSAIDs like ibuprofen has been associated with gastrointestinal complications ranging from stomach irritation to life-threatening GI ulceration bleeding and nephrotoxicity [10,11]. Therefore the development of new NSAIDs without these side effects has long been awaited. Selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs [12]. Thus there remains a compelling need for effective NSAIDs with an improved safety profile. Chronic use of NSAIDs, including ibuprofen, may elicit appreciable GI toxicity [13]; therefore, synthetic approaches based upon NSAIDs chemical modification have been undertaken with the aim of improving the NSAID safety profile. The GI damage from NSAIDs is generally attributed to two factors, local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining the GI health and homeostasis [14]. It has been reported that the derivatization of the carboxyl function of representative NSAIDs, resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect [15,16]. The search for novel analgesic and anti-inflammatory agents devoid of side effects continues to be an active area of research in medicinal chemistry. In fact 1,2,4-triazoles and their derivatives have been reported to possess various biological activities such as anti-inflammatory activity [17] and analgesic properties [18]. Similarly Mannich bases also possess comprehensive bioactivities like anticancer [19], analgesic [20], antibacterial and antifungal activities [21].

Multi-component reactions (MCRs) constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, increased reaction rates to higher yields and

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reproducibility [22]. Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a secondary amine [23]. The aminoalkylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [24]. Similarly Schiff base derivatives of 1,2,4-triazole have displayed good biological activity [25]. So we herein synthesized some novel Schiff bases and Mannich bases carrying both the triazole nucleus as well as ibuprofen moiety and studied their biological properties.

2. Chemistry

The title compounds were synthesized in a one-pot multicomponent Mannich reaction involving ibuprofen triazole (**4**), formaldehyde and secondary amine in ethanol medium. The reaction proceeds via the formation of immonium salt which subsequently attacks the N-1 of triazole giving rise to regioselective Mannich base. It is interesting to note that the reaction is highly regioselective and furnishes only N-Mannich base and none of the S-Mannich derivatives, though the intermediate Schiff base can exist in the thiol–thione tautomeric equilibrium.

Ibuprofen triazole (**4**) was prepared according to the procedure outlined in Scheme 1. Ibuprofen hydrazide (**2**) was obtained by the hydrazinolysis of its corresponding ester. The required dithiocarbazinate (**3**) was synthesized by reacting acid hydrazide with carbon disulphide and potassium hydroxide in ethanol. This salt underwent ring closure on reacting with hydrazine hydrate to give the ibuprofen triazole [26]. Whereas ibuprofen triazole (**4**) was also synthesized in a one-pot reaction, involving the fusion of ibuprofen (**1**) and thiocarbohydrazide (TCH). This alternative one-pot synthesis has matured into a highly useful technique because of higher yield and shorter reaction time. In this one-pot synthesis several disadvantages like long reaction procedure and tedious work-up can be overcome. Due to these facts the one-pot procedure for ibuprofen triazole dominates over conventional Reid and Heindel method [27]. The triazole so synthesized was then condensed with suitable aldehydes in the presence of few drops of conc. Sulphuric acid as a catalyst to yield Schiff bases (**5**) in good yield. Mannich reaction of these Schiff bases was further obtained with formaldehyde and secondary amine in ethanol medium to give the N-Mannich bases (**6**) rather than the S-Mannich bases (Scheme 2). The structures of newly synthesized compounds were confirmed on the basis of spectral, elemental and crystal data. Characterization data of these compounds are given in Tables 1 and 2.

3. Pharmacology

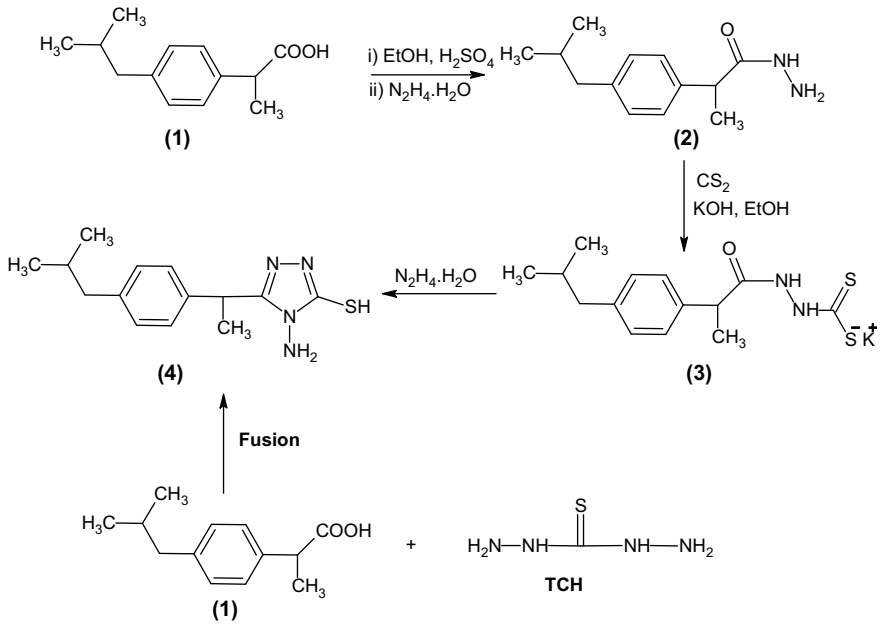
Some of the selected compounds were evaluated for analgesic and anti-inflammatory activities. The test compounds were administered in the form of a suspension (1% carboxy methyl cellulose as vehicle). Anti-inflammatory and analgesic activities of the test compounds were measured with respect to the control and compared with respect to the standard drug; diclofenac. All the pharmacological data are expressed as mean ± SEM; statistical analysis was applied to determine the significance of the difference between the control group and groups of animals treated with the test compounds. Ibuprofen is used as a second reference for comparison of anti-inflammatory activity. Similarly antibacterial and antifungal activity studies were carried out for selected compounds.

3.1. Anti-inflammatory activity

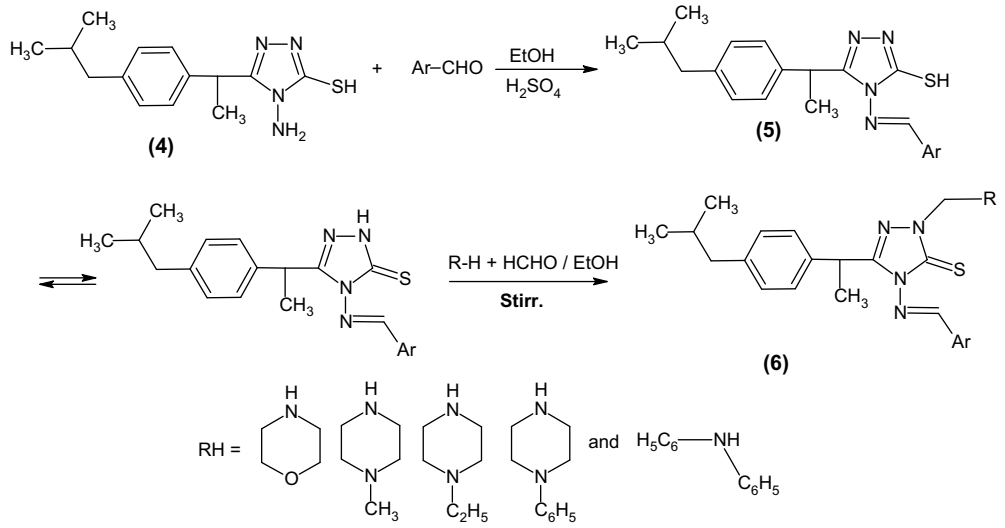
Anti-inflammatory activity was determined by the carrageenan-induced paw edema method in Wistar albino rats by using plethysmography following the method of Winter et al. [28]. Diclofenac at an oral dose of 20 mg/kg served as the standard drug for comparison. The test compounds (20 mg/kg) were administered orally 30 min prior to administration of carrageenan (0.1 mL of 1% w/v) in the plantar region of the paw. The paw volumes were measured at 30, 60, 90, 120, 150 and 180 min after carrageenan administration. The results are presented in Table 3.

3.2. Analgesic activity

Analgesic activities were evaluated on Swiss albino mice by hot plate method. In this method heat is used as a source of pain. Animals are grouped into 5, each containing 6 animals (n = 6).



Scheme 1.



Scheme 2.

Table 1
Characterization data of 4-arylideneamino-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-1,2,4-triazole-3-thiol **5a–f**.

Compounds	Ar	Yield (%)	M.P. (°C)	Mol. formula /(mol. weight)	Analysis (%)	
					Calcd.	Found
5a	C_6H_5	44	140–142	$C_{21}H_{24}N_4S$/(364)	C = 69.23; H = 6.59; N = 15.38; S = 8.79	C = 69.11; H = 6.57; N = 15.42; S = 8.76
5b	4-ClC_6H_4	70	156	$C_{21}H_{24}N_4SCl$/(398.5)	C = 63.23; H = 5.77; N = 14.05; S = 8.03	C = 63.15; H = 5.79; N = 14.02; S = 8.06
5c	2,6-$Cl_2C_6H_3$	54	165–167	$C_{21}H_{22}N_4SCl_2$/(433)	C = 58.19; H = 5.08; N = 12.93; S = 7.39	C = 58.11; H = 5.06; N = 12.89; S = 7.36
5d	4-$CH_3C_6H_4$	85	142	$C_{22}H_{26}N_4S$/(378)	C = 69.84; H = 6.87; N = 14.81; S = 8.46	C = 69.78; H = 6.85; N = 14.77; S = 8.43
5e	4-BrC_6H_4	73	160–164	$C_{21}H_{23}N_4SBr$/(443)	C = 56.88; H = 5.19; N = 12.65; S = 7.22	C = 56.81; H = 5.17; N = 12.60; S = 7.19
5f	4-$NO_2C_6H_4$	78	180–182	$C_{21}H_{23}N_5SO_2$/(409)	C = 61.61; H = 5.62; N = 17.11; S = 7.82	C = 61.51; H = 5.60; N = 17.09; S = 7.79

Animals are individually placed on a hot plate maintained at constant temperature (55 ± 1 °C) and the reaction of animals, such as licking of the paw or jump response is taken as the end point. The reaction time of the animals on the hot plate at 30, 60, 90 min after drug administration (10 mg/kg) is noted. From this percentage increase in reaction time (as index of analgesia) at each time interval is calculated. A dose level of 10 mg/kg diclofenac served as the standard drug for comparison. The test compounds were administered orally by gavage. The responses produced in the animal were observed for 30 min intervals and percentage in reaction[1] time was calculated for analgesic activity using the following equation. Results are presented in Table 4.

$$\%\ \text{increase in reaction time} = \left(\frac{\text{RTAT}}{\text{RTBT}} - 1\right) \times 100$$

where RTAT = Reaction time after treatment; RTBT = Reaction time before treatment.

3.3. Antibacterial and antifungal activities

Well diffusion method was employed for the determination of antibacterial and antifungal activities. *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram −ve) are the bacteria used for the antibacterial assay. Similarly *Aspergillus* was used for the antifungal assay. Compounds in solution form, i.e. of concentration 1000 μg/mL (10 mg of compound dissolved in 10 mL DMF) was assayed for antimicrobial activities after sterilizing by autoclaving. These standard solutions were added to the well, digged using a borer and incubated at 37 °C for 24–48 hours and observed. But none of the compounds showed any significant antibacterial or antifungal activity compared with the standard drugs.

4. Results and discussion

4.1. Pharmacological screening

The anti-inflammatory activity of the target compounds **5** and **6** was evaluated by applying the carrageenan-induced rat paw edema bioassay in rats. Diclofenac was used as a reference substance in the assay. For comparison purposes, ibuprofen was additionally used as a second standard reference. All the tested compounds were found more potent than ibuprofen. In comparison to diclofenac, all the test compounds showed comparable anti-inflammatory activity (Table 3). Mannich base (**6b**) displayed the highest anti-inflammatory activity among the set of compounds tested in the present study. Test compounds that exhibited the most potent anti-inflammatory activity namely **6b**, **6f**, **6k** and **6l** were further evaluated for their analgesic activity in mice. In general, the tested compounds showed a better analgesic activity compared to diclofenac as illustrated in Table 4.

Some of the synthesized compounds **5** and **6** were tested for their anti-inflammatory activity at an equimolar oral dose relative to 20 mg/kg diclofenac and ibuprofen. The compounds showed anti-inflammatory activity ranging from 56% to 74% (Table 3), whereas standard drugs diclofenac and ibuprofen showed 65% and 42% inhibition after 3 hours. The anti-inflammatory activity data

[1] Maximum tolerated reaction time is used for calculation.

Table 2
Characterization data of 4-arylideneamino-2-substituted aminomethyl-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-3*H*-1,2,4-triazole-3-thione **6a–n**.

Compounds	Ar	R	Yield (%)/ M.P. (°C)	Mol. formula/ (mol. weight)	Analysis (%)	
					Calcd.	Found
6a	C_6H_5	Morpholine	92/69–71	$C_{26}H_{33}N_5OS$/(463)	C = 67.38; H = 7.12; N = 15.11; S = 6.91	C = 67.24; H = 7.10; N = 15.06; S = 6.89
6b	4-ClC_6H_4	Morpholine	90/100–102	$C_{26}H_{32}N_5SOCl$/(497.5)	C = 62.71; H = 6.43; N = 14.07; S = 6.43	C = 62.62; H = 6.45; N = 14.10; S = 6.45
6c	4-ClC_6H_4	1-Methylpiperazine	63/115	$C_{27}H_{35}N_6SCl$/(510.5)	C = 63.46; H = 6.85; N = 16.45; S = 6.26	C = 63.31; H = 6.88; N = 16.40; S = 6.24
6d	2,6-$Cl_2C_6H_3$	Morpholine	80/70	$C_{26}H_{31}N_5SOCl_2$/(532)	C = 58.64; H = 5.82; N = 13.15; S = 6.01	C = 58.49; H = 5.80; N = 13.12; S = 5.99
6e	2,6-$Cl_2C_6H_3$	1-Phenylpiperazine	81/104–107	$C_{32}H_{36}N_6SCl_2$/(607)	C = 63.26; H = 5.93; N = 13.83; S = 5.27	C = 63.18; H = 5.95; N = 13.78; S = 5.25
6f	4-$CH_3C_6H_4$	Morpholine	63/61–63	$C_{27}H_{35}N_5SO$/(477)	C = 67.92; H = 7.33; N = 14.67; S = 6.70	C = 67.81; H = 7.36; N = 14.63; S = 6.68
6g	4-$CH_3C_6H_4$	1-Methylpiperazine	47/65	$C_{28}H_{38}N_6S$/(490)	C = 68.57; H = 7.75; N = 17.14; S = 6.53	C = 68.42; H = 7.78; N = 17.08; S = 6.55
6h	4-$CH_3C_6H_4$	Diphenylamine	89/116	$C_{35}H_{37}N_5S$/(559)	C = 75.13; H = 6.61; N = 12.52; S = 5.72	C = 75.02; H = 6.59; N = 12.48; S = 5.74
6i	4-$CH_3C_6H_4$	1-Phenylpiperazine	88/104	$C_{33}H_{40}N_6S$/(552)	C = 71.73; H = 7.24; N = 15.21; S = 5.79	C = 71.61; H = 7.21; N = 15.18; S = 5.81
6j	4-BrC_6H_4	Diphenylamine	68/108–109	$C_{34}H_{34}N_5SBr$/(624)	C = 65.38; H = 5.44; N = 11.21; S = 5.12	C = 65.23; H = 5.42; N = 11.18; S = 5.10
6k	4-$NO_2C_6H_4$	Morpholine	87/114	$C_{26}H_{32}N_6SO_3$/(508)	C = 61.41; H = 6.29; N = 16.53; S = 6.29	C = 61.34; H = 6.27; N = 16.48; S = 6.31
6l	4-$NO_2C_6H_4$	1-Methylpiperazine	87/116–117	$C_{27}H_{35}N_7SO_2$/(521)	C = 62.18; H = 6.71; N = 18.80; S = 6.14	C = 62.07; H = 6.69; N = 18.75; S = 6.16
6m	4-$NO_2C_6H_4$	Diphenylamine	95/95–96	$C_{34}H_{34}N_6SO_2$/(590)	C = 69.15; H = 5.76; N = 14.23; S = 5.42	C = 69.08; H = 5.75; N = 14.19; S = 5.44
6n	4-$NO_2C_6H_4$	1-Ethylpiperazine	52/200–02	$C_{28}H_{37}N_7SO_2$/(535)	C = 62.80; H = 6.91; N = 18.31; S = 5.98	C = 62.71; H = 6.88; N = 18.26; S = 5.96

showed that the compounds having morpholine group (**6b**) and methylpiperazine (**6l**) possess highest activity (74%). Among the Schiff bases tested for anti-inflammatory activity, **5e** showed 74% activity and **5b** showed 64% activity. On aminomethylation of **5b**, by morpholine, anti-inflammatory activity changes to 74%. Replacement of morpholine by methylpiperazine resulted in a slight increase of activity (74%, $p > 0.05$). Further among Mannich bases, **6k** showed 73% activity. Similarly **6f** showed good anti-inflammatory activity (68%, $p > 0.05$). The compounds that showed anti-inflammatory activity higher than 70% were further tested for their analgesic activity at an equimolar oral dose relative to 10 mg/kg diclofenac. Compounds **6b**, **6f**, **6k** and **6l** showed analgesic activity ranging from 64.32% to 79.6%, whereas the standard drug diclofenac showed 79.24% inhibition. It was noted that the compounds showing highest anti-inflammatory activity also exhibited highest analgesic activity. Among these compounds, **6k** showed activity (79.60%, $p > 0.05$) more than that of standard diclofenac, whereas compounds **6b** (76.49%, $p > 0.01$), **6f** (64.32%, $p > 0.05$) and **6l** (70.75%, $p > 0.01$) showed good analgesic activity.

For further comparison, a graph was plotted showing the anti-inflammatory activities of **4**, **5b**, **6b** with ibuprofen (Fig. 1). This graph indicates that all the ibuprofen analogues including its triazole, Schiff and Mannich bases showed excellent anti-inflammatory activity than ibuprofen at every time interval. Among these, Mannich derivative of ibuprofen showed the highest activity.

5. Conclusion

It has been speculated that ibuprofen acts non-selectively probably because of its relatively small size. It appears that bulkier structures are more likely to confer selectivity due to its wider active site. Thus it seems that the conversion of carboxylic group of ibuprofen to triazole and its Schiff and Mannich derivatives might have caused good inhibitory activity, resulting in significant analgesic and anti-inflammatory activities. On the basis of the results obtained, ibuprofen triazole, Schiff and Mannich bases appear to be suitable moiety in the field of medicine.

It could be concluded that replacement of the carboxyl function of ibuprofen by certain bulkier moieties generally improves their pharmacological profile. It was interesting to note that all the non-carboxylic test compounds were found to have anti-inflammatory activity greater than that of ibuprofen. More interestingly, some of the test compounds were more potent than diclofenac.

Table 3
Anti-inflammatory activity data of ibuprofen derivatives.

Compounds	(% Inhibition ± SEM)[a]					
	0.5 hours	1 hours	1.5 hours	2 hours	2.5 hours	3 hours
Diclofenac	83 ± 2.38[b]	59 ± 1.01[b]	70 ± 2.56[c]	87 ± 4.18[c]	69 ± 3.9[b]	65 ± 0.89[c]
Ibuprofen	20 ± 4.31	18 ± 2.67[b]	25 ± 2.69[b]	36 ± 3.74[b]	39 ± 4.25[c]	42 ± 2.35[b]
4	30 ± 1.97[b]	32 ± 3.64	46 ± 1.88[b]	45 ± 4.08	50 ± 1.26	56 ± 3.38[b]
5b	62 ± 5.02	49 ± 2.11	52 ± 4.14	57 ± 4.41[b]	62 ± 0.79	64 ± 4.1[b]
5e	0	16 ± 1.79	65 ± 1.74[b]	60 ± 1.15	70 ± 1.99[b]	72 ± 2.19[b]
6b	90 ± 2.27[b]	42 ± 1.39[b]	61 ± 4.27[b]	61 ± 4.29[c]	73 ± 5.31	74 ± 0.77[c]
6f	0	23 ± 4.08	64 ± 4.16[b]	60 ± 3.72	67 ± 3.31[c]	68 ± 3.79[b]
6j	9 ± 3.87	10 ± 4.42	38 ± 1.48	38 ± 1.29	57 ± 2.84[b]	57 ± 4.28
6k	12 ± 1.09[b]	52 ± 1.93	65 ± 3.92[b]	54 ± 3.28	71 ± 2.07[b]	73 ± 1.56
6l	38 ± 3.23	70 ± 2.98[b]	82 ± 2.59	68 ± 3.08[b]	75 ± 2.8	74 ± 1.78[c]

[a] Results are expressed as their mean values (n = 6).
[b] $P < 0.05$.
[c] $P < 0.01$; significant from the control.

6. Experimental protocols

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded by dispersing the compounds in KBr pellets on a Shimadzu FT-IR 157 spectrometer. ^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance II 400 NMR spectrophotometer and all the chemical shift values were reported as δ (ppm). ^{13}C NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer. Mass spectra were recorded on a JEOL SX-102 (FAB) mass spectrometer.

*6.1. General procedure for the synthesis of 4-amino-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-1,2,4-triazole-3-thiol **4***

An equimolar mixture of thiocarbohydrazide (TCH) and ibuprofen (**1**) was mixed and heated gently on an oil bath until the evolution of H_2S ceases. The reaction mixture was then cooled to room temperature and poured into ice cold water and stirred well. It was then filtered, dried and recrystallized from ethanol.

Table 4
Analgesic activity data of **6b**, **6f**, **6k** & **6l**.

Compounds	Before treatment ± SEM	After treatment ± SEM (1.5 hours)	% Increase in reaction time ± SEM[a]
Diclofenac	2.12 ± 0.18	3.80 ± 0.16	79.24[b]
6b	2.27 ± 0.26	4.01 ± 0.27	76.49[c]
6f	2.49 ± 0.29	4.10 ± 0.22	64.32[b]
6k	2.02 ± 0.17	3.63 ± 0.16	79.60[b]
6l	2.50 ± 0.26	4.21 ± 0.19	70.75[c]

[a] Results are expressed as their mean values (n = 6).
[b] $P < 0.05$.
[c] $P < 0.01$; significant from the control.

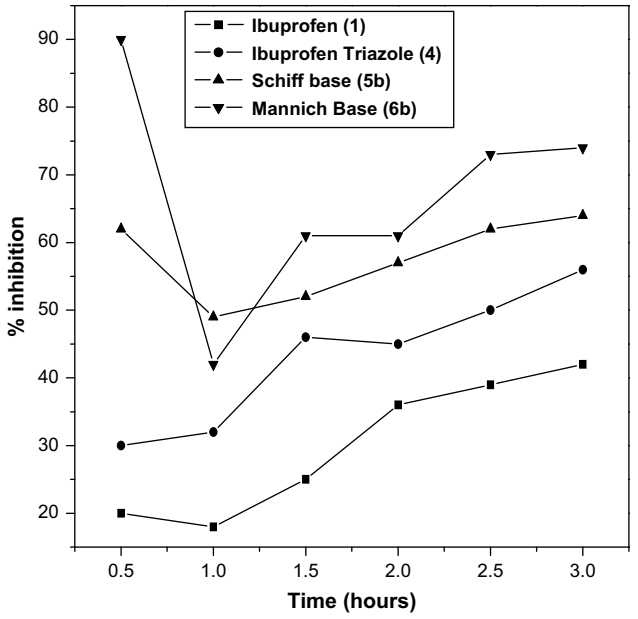


Fig. 1. Anti-inflammatory activity of ibuprofen and its derivatives at 20 mg/kg (molar equivalent to ibuprofen).

IR (KBr) γ/cm^{-1}: 3319 (N–H), 2866 (C–H), 1600 (C═N), 1292 (C═S); ^1H NMR (DMSO-d_6) δ: 0.85 (d, 6H, $J = 6.57$ Hz, $(CH_3)_2$), 1.53 (d, 3H, $J = 7.20$ Hz, CH_3), 1.72–1.85 (m, 1H, CH–$(CH_3)_2$), 2.40 (d, 2H, $J = 7.18$ Hz, CH_2), 4.33 (q, 1H, $J = 7.20$ Hz, CH–CH_3), 5.40 (s, 2H, NH_2), 7.10 (d, 2H, $J = 7.90$ Hz, 3′,5′-H), 7.15 (d, 2H, $J = 7.90$ Hz, 2′,6′-H), 13.56 (s, 1H, SH/NH); FABMS (m/z, %): 277 ($M^+ + 1$, 89), 276 (M^+, 23), 261 (67), 188 (35), 161 (100).

6.2. General procedure for the synthesis of 4-arylideneamino-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-1,2,4-triazole-3-thiol **5**

A mixture of 4-amino-5-[1(4-iso-butylphenyl)ethyl]-3-mercapto-1,2,4-triazole (**4**) (10 mmol), substituted benzaldehydes (10 mmol) and 2–3 drops of concentrated sulphuric acid in ethanol medium was heated to reflux for 3 hours. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol. The characterization data of these compounds are given in Table 1.

6.2.1. Compound ***5b***

IR (KBr) γ/cm^{-1}: 3134 (N–H), 2949 (C–H), 1570 (C═N), 1280 (C═S); ^1H NMR ($CDCl_3$) δ: 0.83 (d, 6H, $J = 6.60$ Hz, $(CH_3)_2$), 1.67 (d, 3H, $J = 7.20$ Hz, CH_3), 1.78–1.852 (m, 1H, CH–$(CH_3)_2$), 2.39 (d, 2H, $J = 7.20$ Hz, CH_2), 3.94 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 7.04 (d, 2H, $J = 8.10$ Hz, 3′,5′-H), 7.09 (d, 2H, $J = 8.10$ Hz, 2′,6′-H), 7.65 (d, 2H, $J = 8.80$ Hz, 2″,6″-H), 8.02 (d, 2H, $J = 8.80$ Hz, 3″,5″-H), 10.09 (s, 1H, N═CH), 10.53 (s, 1H, SH/NH); FABMS (m/z, %): 400 ($M^+ + 2$, 89), 399 ($M^+ + 1$, 82), 398 (M^+, 100), 261 (67), 161 (18).

6.2.2. Compound ***5e***

IR (KBr) γ/cm^{-1}: 3132 (N–H), 2927 (C–H), 1608 (C═N), 1284 (C═S); ^1H NMR ($CDCl_3$) δ: 0.80 (d, 6H, $J = 6.61$ Hz, $(CH_3)_2$), 1.61 (d, 3H, $J = 7.20$ Hz, CH_3), 1.728–1.806 (m, 1H, CH–$(CH_3)_2$), 2.37 (d, 2H, $J = 7.20$ Hz, CH_2), 4.03 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 7.06 (d, 2H, $J = 8.12$ Hz, 3′,5′-H), 7.12 (d, 2H, $J = 8.10$ Hz, 2′,6′-H), 7.76 (d, 2H, $J = 8.80$ Hz, 2″,6″-H), 8.10 (d, 2H, $J = 8.80$ Hz, 3″,5″-H), 10.11 (s, 1H, N═CH), 10.67 (s, 1H, SH/NH); FABMS (m/z, %): 444 ($M^+ + 2$, 100), 442 (M^+, 99), 261 (100), 161 (10).

6.2.3. Compound ***5f***

IR (KBr) γ/cm^{-1}: 3133 (N–H), 2942 (C–H), 1600 (C═N), 1283 (C═S); ^1H NMR ($CDCl_3$) δ: 0.81 (d, 6H, $J = 6.58$ Hz, $(CH_3)_2$), 1.70 (d, 3H, $J = 7.20$ Hz, CH_3), 1.75–1.81 (m, 1H, CH–$(CH_3)_2$), 2.39 (d, 2H, $J = 7.12$ Hz, CH_2), 4.20 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 7.03 (d, 2H, $J = 8.10$ Hz, 3′,5′-H), 7.11 (d, 2H, $J = 8.10$ Hz, 2′,6′-H), 7.65 (d, 2H, $J = 8.82$ Hz, 2″,6″-H), 8.12 (d, 2H, $J = 8.82$ Hz, 3″,5″-H), 10.23 (s, 1H, N═CH), 10.89 (s, 1H, SH/NH); FABMS (m/z, %): 410 ($M^+ + 1$, 100), 409 (M^+, 18), 161 (27).

6.3. General procedure for the synthesis of 4-arylideneamino-2-substituted aminomethyl-5-{1-[4-(2-methylpropyl)phenyl]ethyl}-3H-1,2,4-triazole-3-thione **6**

A mixture of Schiff bases (**5**) (10 mmol), formaldehyde (40%, 1.5 mL) and appropriate secondary-amines (10 mmol) in ethanol medium was stirred at room temperature for 12 hours. The precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol. The characterization data of these compounds are given in Table 2. In the ^{13}C NMR assignment for compounds **6h**, aryl carbons showed characteristic signals at 117.85–146.59 ppm and 161.94 (C═S) and **6j**, aryl carbons showed characteristic signals at 117.84–140.51 ppm and 161.53 ppm (C═S) (Fig. 2)

6.3.1. Compound ***6f***

IR (KBr) γ/cm^{-1}: 2967, 2863 (C–H), 1615 (C═N), 1261 (C═S); ^1H NMR ($CDCl_3$) δ: 0.83 (d, 6H, $J = 6.60$ Hz, $(CH_3)_2$), 1.70 (d, 3H, $J = 7.28$ Hz, CH_3), 1.72–1.79 (m, 1H, CH–$(CH_3)_2$), 2.39 (d, 2H, $J = 7.16$ Hz, CH_2), 2.41 (s, 3H, C_6H_4–CH_3), 2.86 (t, 4H, CH_2NCH_2), 3.72 (t, 4H, CH_2OCH_2), 4.39 (q, 1H, $J = 7.28$ Hz, $CHCH_3$), 5.17 (s, 2H, NCH_2N), 7.02 (d, 2H, $J = 8.12$ Hz, 3″,5″-H), 7.14 (d, 2H, $J = 8.08$ Hz, 3′,5′-H), 7.24 (d, 2H, $J = 8.08$ Hz, 2′,6′-H), 7.63 (d, 2H, $J = 8.12$ Hz, 2″,6″-H), 9.88 (s, 1H, N═CH); FABMS (m/z, %): 478 ($M^+ + 1$, 57), 477 (M^+, 32), 161 (76), 100 (100).

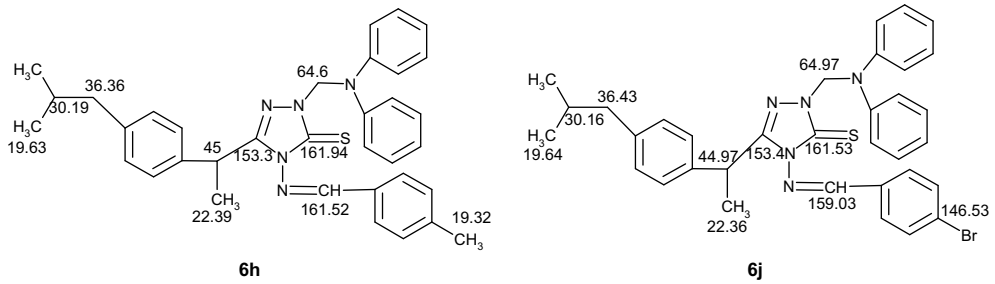


Fig. 2. ^{13}C NMR assignment of some carbon atoms of compounds **6h** and **6j**.

6.3.2. Compound ***6h***

IR (KBr) γ/cm^{-1}: 2970, 2859 (C–H), 1611 (C═N), 1254 (C═S); ^1H NMR ($CDCl_3$) δ: 0.81 (d, 6H, $J = 6.42$ Hz, $(CH_3)_2$), 1.61 (d, 3H, $J = 7.24$ Hz, CH_3), 1.67–1.78 (m, 1H, CH–$(CH_3)_2$), 2.37 (d, 2H, $J = 7.16$ Hz, CH_2), 2.39 (s, 3H, C_6H_4–CH_3), 4.32 (q, 1H, $J = 7.24$ Hz, $CHCH_3$), 6.09 (s, 2H, NCH_2N), 6.93–7.64 (m, 14H, Ar–H), 6.95 (d, 2H, $J = 8.12$ Hz, 3″,5″-H), 7.61 (d, 2H, $J = 8.12$ Hz, 2″,6″-H), 9.92 (s, 1H, N═CH); FABMS (m/z, %): 560 ($M^+ + 1$, 52), 559 (M^+, 46), 182 (100), 161 (95), 118 (38).

6.3.3. Compound ***6j***

IR (KBr) γ/cm^{-1}: 2960, 2854 (C–H), 1608 (C═N), 1263 (C═S); ^1H NMR ($CDCl_3$) δ: 0.81 (d, 6H, $J = 6.58$ Hz, $(CH_3)_2$), 1.61 (d, 3H, $J = 7.20$ Hz, CH_3), 1.68–1.76 (m, 1H, CH–$(CH_3)_2$), 2.37 (d, 2H, $J = 7.20$ Hz, CH_2), 4.31 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 6.08 (s, 2H, NCH_2N), 6.94–7.57 (m, 18H, Ar–H), 10.17 (s, 1H, N═CH); FABMS (m/z, %): 623 ($M^+ + 2$, 85), 624 ($M^+ + 1$, 89), 623 (M^+, 78), 441 (59), 440 (29), 182 (100), 161 (27).

6.3.4. Compound ***6k***

IR (KBr) γ/cm^{-1}: 2951, 2868 (C–H), 1610 (C═N), 1264 (C═S); ^1H NMR ($CDCl_3$) δ: 0.82 (d, 6H, $J = 6.60$ Hz, $(CH_3)_2$), 1.72 (d, 3H, $J = 7.24$ Hz, CH_3), 1.73–1.79 (m, 1H, CH–$(CH_3)_2$), 2.40 (d, 2H, $J = 7.12$ Hz, CH_2), 2.87 (t, 4H, CH_2NCH_2), 3.73 (t, 4H, CH_2OCH_2), 4.42 (q, 1H, $J = 7.24$ Hz, $CHCH_3$), 5.18 (s, 2H, NCH_2N), 7.06 (d, 2H, $J = 8.12$ Hz, 3′,5′-H), 7.13 (d, 2H, $J = 8.12$ Hz, 2′,6′-H), 7.86 (d, 2H, $J = 8.84$ Hz, 2″,6″-H), 8.28 (d, 2H, $J = 8.84$ Hz, 3″,5″-H), 10.65 (s, 1H, N═CH); FABMS (m/z, %): 509 ($M^+ + 1$, 68), 508 (M^+, 25), 161 (86), 100 (100).

6.3.5. Compound ***6l***

IR (KBr) γ/cm^{-1}: 2953, 2858 (C–H), 1607 (C═N), 1273 (C═S); ^1H NMR ($CDCl_3$) δ: 0.81 (d, 6H, $J = 6.60$ Hz, $(CH_3)_2$), 1.71 (d, 3H, $J = 7.20$ Hz, CH_3), 1.72–1.79 (m, 1H, CH–$(CH_3)_2$), 2.29 (s, 3H, N–CH_3), 2.39 (d, 2H, $J = 7.12$ Hz, CH_2), 2.46 (t, 4H, CH_2NCH_2), 2.95 (t, 4H, CH_2NCH_2), 4.38 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 5.28 (s, 2H, NCH_2N), 7.04 (d, 2H, $J = 8.12$ Hz, 3′,5′-H), 7.12 (d, 2H, $J = 8.10$ Hz, 2′,6′-H), 7.84 (d, 2H, $J = 8.84$ Hz, 2″,6″-H), 8.27 (d, 2H, $J = 8.84$ Hz, 3″,5″-H), 10.62 (s, 1H, N═CH); FABMS (m/z, %): 522($M^+ + 1$, 55), 521 (M^+, 41), 161 (78), 113 (100).

6.3.6. Compound ***6m***

IR (KBr) γ/cm^{-1}: 2961, 2855 (C–H), 1601 (C═N), 1255 (C═S); ^1H NMR ($CDCl_3$) δ: 0.81 (d, 6H, $J = 6.58$ Hz, $(CH_3)_2$), 1.62 (d, 3H, $J = 7.20$ Hz, CH_3), 1.69–1.77 (m, 1H, CH–$(CH_3)_2$), 2.39 (d, 2H, $J = 7.18$ Hz, CH_2), 4.32 (q, 1H, $J = 7.20$ Hz, $CHCH_3$), 5.98 (s, 2H, NCH_2N), 7.02 (d, 2H, $J = 8.08$ Hz, 3′,5′-H), 7.12 (d, 2H, $J = 8.08$ Hz, 2′,6′-H), 7.62 (d, 2H, $J = 8.82$ Hz, 2″,6″-H), 7.86 (d, 2H, $J = 8.82$ Hz, 3″,5″-H), 10.32 (s, 1H, N═CH); FABMS (m/z, %): 591($M^+ + 1$, 84), 590 (M^+, 81), 182 (100), 161 (55).

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