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Synthesis and biological activities of some triazolothiadiazoles containing ibuprofen moiety

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ABSTRACT

In the present study, a new series of 6-(5-aryl-2-furyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (**7a-l**) were synthesized by the condensation of 4-amino-5-[1-(4-isobutylphenyl)ethyl]-3-mercapto-[1,2,4]-triazole (**6**) with various substituted aryl furoic acids in good yield. The newly synthesized compounds were confirmed on the basis of elemental analyses, IR, ¹H NMR and Mass spectral data. All compounds were screened for their antibacterial and anti-inflammatory activity. Among the synthesized compounds **7a**, **7b**, **7d**, **7e**, **7f**, **7g** and **7l** exhibited good antibacterial activity and compounds **7b**, **7h**, **7i** and **7l** exhibited very good anti-inflammatory activity.

Keywords: [1,2,4]-triazoles, [1,3,4]-thiadiazoles, [1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles, antibacterial, anti-inflammatory.

INTRODUCTION

In the recent years [1,2,4]-triazoles have captured the attention owing to their effective application as drugs in the treatment of various diseases. [1,2,4]-triazole derivatives are known to possess broad spectrum of biological activities such as antimicrobial and anticancer [1], antitubercular [2], analgesic and anti-inflammatory [3] and antiviral [4]. Mainly 1,2,4-triazole derivatives are successfully marketed as broad spectrum antifungal drugs such as fluconazole, voriconazole and itraconazole. Moreover several other 1,2,4-triazole derivatives used as drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent) and Alprazolam (anxiolytic agent).

[1,3,4]-Thiadiazole is a versatile moiety that exhibits wide variety of biological activities. The earliest use of thiadiazoles was in pharmaceutical area, as antibacterials with properties similar to those of sulphonamide drugs. Later they were found to possess diverse biological activities like anticancer [5], antiproliferative [6], antioxidant [7], antidepressant [8], antitrypanosomal [9], carbonic anhydrase inhibitory activity [10] and leishmanicidal activity [11]. The extensive application of these two scaffolds in drug designing has made us to synthesize some newer molecules comprising these two units. Triazolothiadiazoles have been reported for their ulcerogenic activity [12], antimicrobial [13], antitumor [14], anti-inflammatory [15], antitubercular [16] and CNS depressant [17]. And also they have been reported in agriculture field as insecticides [18].

Ibuprofen is an arylpropionic acid class of non steroidal anti-inflammatory drug which is widely used in the treatment of inflammation and pain. However, these drugs are known to provoke adverse effects such as ulceration,

gastrointestinal bleeding and heart attack [19]. The reported literature confirms that gastrointestinal side effects of arylpropionic acids are due to the presence of a free carboxylic group in the parent drug [20-22]. Hence, the search for novel anti-inflammatory drug is gaining importance in the recent years. The replacement of the terminal carboxylic function of propionic acid by oxadiazole ring may enhance the anti-inflammatory activity of such compounds with reduced ulcerogenic effects [23]. Ibuprofen derived heterocycles have found to be associated with different activity such as antimicrobial, analgesic along with anti-inflammatory activity [24, 25]. Owing to the pharmacological importance of 1,2,4-triazole and 1,3,4-thiadiazole moieties and in continuation our work on heterocycles derived from ibuprofen [26-29] and aryl furan moiety [30, 31], it was planned to synthesize some new triazolothiadiazoles derivatives of ibuprofen having aryl furansubstituents and to study their biological properties.

MATERIALS AND METHODS

Chemistry

The melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were recorded ($\text{CDCl}_3/\text{DMSO}-d_6$ mixture) on a BRUKER AVANCE II-400 spectrometer at 400 MHz and 100 MHz respectively using TMS as an internal standard. Mass spectra were recorded in Agilent Technology LC-mass spectrometer. Elemental analyses (CHNS) were performed on the CHNS Elementar Vario EL III. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates.

General Procedure for the Preparation of Potassium Dithiocarbazinate (5)

To a continuously stirred solution of potassium hydroxide (8.4 g, 0.15 mol) and hydrazide (4) (18.5 g, 0.1 mol) in absolute ethanol (100 mL), carbon disulphide (11.2 g, 0.15 mol) was added dropwise. After the addition, the mixture was diluted with absolute ethanol (75 mL) and agitated for 16 h. It was then diluted with dry ether (100 mL) and the precipitated solid was collected by filtration, washed with ether and dried at 65°C under vacuum. The potassium salt was obtained in quantitative yield and was used for next reaction without any further purification.

Procedure for the Preparation of Substituted Arylfuroic Acids (6)

A mixture containing substituted aniline (100 mmol), hydrochloric acid (15%, 60 mL) and water 90 mL was heated until a clear solution was obtained, cooled to 0°C , diazotized with aqueous sodium nitrite (30%, 24 mL) and filtered. To the filtered solution, water (50 mL) and furoic acid (9.6g, 100 mmol) were added. Aqueous solution of cupric chloride (2.5g in 10 mL of water) was added drop wise and stirred for four hours at room temperature and kept aside for 16 h. The resulting solid was filtered off, suspended in water and purified by steam distillation. These compounds were then recrystallized from ethanol.

General Procedure for the Preparation of 4-Amino-5-[1-(4-isobutylphenyl)ethyl]-3-mercapto-[1,2,4]-triazole (6)

To a suspension of potassium dithiocarbazinate (5) (0.1 mol), hydrazine hydrate (10 mL, 0.2 mol) was added. The reaction mixture was refluxed for about an hour. The colour of the reaction mixture changed to green, with the evolution of hydrogen sulphide and a homogeneous mass was obtained. Reaction mixture was cooled and diluted with cold water (100 mL), acidified with concentrated hydrochloric acid. The solid product obtained was filtered, washed with water, dried and recrystallized from methanol. M.p. 153°C ; Yield 83 %.

General Procedure for the Preparation of 6-(5-Aryl-2-furyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazoles (7a-l)

A mixture of 4-amino-5-[1-(4-isobutylphenyl)ethyl]-3-mercapto-[1,2,4]-triazole (6) (0.01 mol), various substituted arylfuroic acids (0.01 mol) and phosphorus oxychloride (10 mL) was refluxed for 8 hours on a water bath. Excess of phosphorous oxychloride was removed under vacuum. The thick mass obtained was treated with water and left overnight. Solid product thus obtained was filtered, washed with 2% sodium bicarbonate solution, then with water, dried and recrystallized with appropriate solvent.

6-[5-(2-Chlorophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (7a)

IR (KBr, γ_{max} , cm^{-1}): 3094 (ArC-H), 2955 (C-H), 1630 (C=N), 1529 (C=C), 739 (C-Cl). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 0.84 (d, 6H, CH_3 , $J = 8$ Hz), 1.68 (d, 3H, CH_3 , $J = 8$ Hz), 1.81-1.85 (m, 1H, CH), 2.46 (d, 2H, CH_2 , $J = 8$ Hz), 4.40 (q, 1H, CH, $J = 8$ Hz), 6.89 (d, 1H, furan H, $J = 3.6$ Hz), 7.04 (d, 1H, furan H, $J = 3.6$ Hz), 7.13 (d, 2H, 4-isobutylphenyl, $J = 8.4$ Hz), 7.28 (d, 2H, isobutylphenyl, $J = 8$ Hz), 7.28-7.44 (m, 4H, 2-chlorophenyl). LC MS: m/z (%) = 463 (M+1), 465 (M+3)

6-[5-(4-Chlorophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (7b)

IR (KBr, γ_{max} , cm^{-1}): 3094 (ArC-H), 2955 (C-H), 1630 (C=N), 1529 (C=C), 739 (C-Cl). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 0.84 (d, 6H, CH_3 , $J = 8$ Hz), 1.68 (d, 3H, CH_3 , $J = 8$ Hz), 1.81-1.85 (m, 1H, CH), 2.46 (d, 2H, CH_2 , $J = 8$

H_z), 4.40 (q, 1H, CH, *J* = 8 Hz), 6.87 (d, 1H, furan H, *J* = 3.6 Hz), 7.00 (d, 1H, furan H, *J* = 3.6 Hz), 7.11 (d, 2H, 4-isobutylphenyl, *J* = 8.4 Hz), 7.25 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.28 (d, 2H, 4-chlorophenyl, *J* = 8 Hz), 7.62 (d, 2H, 4-chlorophenyl, *J* = 8 Hz). LC MS: *m/z* (%) = 463 (M+1, 54%), 465 (M+3)

6-[5-(4-Bromophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (7c)

IR (KBr, γ_{\max} , cm⁻¹): 3098 (ArC-H), 2986 (C-H), 1630 (C=N), 1513 (C=C), 882 (C-Cl). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.84 (d, 6H, CH₃, *J* = 8 Hz), 1.76 (d, 3H, CH₃, *J* = 8 Hz), 1.80-1.85 (m, 1H, CH), 2.43 (d, 2H, CH₂, *J* = 8 Hz), 4.32 (q, 1H, CH, *J* = 7.6 Hz), 6.86 (d, 1H, furan H, *J* = 4 Hz), 7.01 (d, 1H, furan H, *J* = 4 Hz), 7.11 (d, 2H, isobutylphenyl, *J* = 8.4 Hz), 7.31 (d, 2H, isobutylphenyl, *J* = 4.8 Hz), 7.41 (d, 2H, 4-bromophenyl, *J* = 8 Hz), 7.66 (d, 2H, 4-bromophenyl, *J* = 8.8 Hz). LC MS: *m/z* (%) = 507 (M+1), 509 (M+3)

6-[5-(4-Methylphenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (7d)

IR (KBr, γ_{\max} , cm⁻¹): 3087 (ArC-H), 2950 (C-H), 1625 (C=N), 1520 (C=C). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.85 (d, 6H, CH₃, *J* = 8 Hz), 1.68 (d, 3H, CH₃, *J* = 8 Hz), 1.81-1.85 (m, 1H, CH), 2.42 (s, 3H, Ar-CH₃), 2.46 (d, 2H, CH₂, *J* = 8 Hz), 4.40 (q, 1H, CH, *J* = 8.0 Hz), 6.84 (d, 1H, furan H, *J* = 3.6 Hz), 6.96 (d, 1H, furan H, *J* = 3.6 Hz), 7.14 (d, 2H, ibuprofen, *J* = 8.0 Hz), 7.25 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.19 (d, 2H, 4-methylphenyl, *J* = 8 Hz), 7.32 (d, 2H, 4-methylphenyl, *J* = 8 Hz). LC MS: *m/z* (%) = 443 (M+1)

6-[5-(2-trifluoromethylphenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-b] [1,3,4]-thiadiazoles (7e)

IR (KBr, γ_{\max} , cm⁻¹): 3092 (ArC-H), 2945 (C-H), 1629 (C=N), 1527 (C=C), 1080 (C-F). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.87 (d, 6H, CH₃, *J* = 8 Hz), 1.69 (d, 3H, CH₃, *J* = 8 Hz), 1.83-1.87 (m, 1H, CH), 2.40 (s, 3H, Ar-CH₃), 2.44 (d, 2H, CH₂, *J* = 8 Hz), 4.45 (q, 1H, CH, *J* = 8.0 Hz), 6.86 (d, 1H, furan H, *J* = 3.6 Hz), 7.06 (d, 1H, furan H, *J* = 3.6 Hz), 7.11 (d, 2H, ibuprofen, *J* = 8.0 Hz), 7.23 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.51-7.81 (m, 4H, 2-trifluoromethylphenyl, *J* = 8 Hz). LC MS: *m/z* = 497 (M+1)

6-[5-(2-Nitrophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazoles (7f)

IR (KBr, γ_{\max} , cm⁻¹): 3086 (ArC-H), 2948 (C-H), 1629 (C=N), 1519 (C=C), 1528 (NO₂ asymmetric stretch), 1334 (NO₂ symmetric stretch). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.85 (d, 6H, CH₃, *J* = 8 Hz), 1.68 (d, 3H, CH₃, *J* = 8 Hz), 1.81-1.85 (m, 1H, CH), 2.46 (d, 2H, CH₂, *J* = 8.0 Hz), 4.40 (q, 1H, CH, *J* = 8 Hz), 6.99 (d, 1H, furan H, *J* = 4 Hz), 7.05 (d, 1H, furan H, *J* = 4 Hz), 7.13 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.26 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.58-7.70 (m, 3H, 2-nitrophenyl), 7.82 (d, 1H, 2-nitrophenyl, *J* = 8 Hz). LC MS: *m/z* = 474 (M+1)

6-[5-(4-nitrophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b] [1,3,4]-thiadiazoles (7g)

IR (KBr, γ_{\max} , cm⁻¹): 3092 (ArC-H), 2950 (C-H), 1634 (C=N), 1525 (C=C), 1540 (asymmetric stretch), 1334 (NO₂ symmetric stretch). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.85 (d, 6H, CH₃, *J* = 8 Hz), 1.65 (d, 3H, CH₃, *J* = 8 Hz), 1.82-1.86 (m, 1H, CH), 2.45 (d, 2H, CH₂, *J* = 8.0 Hz), 4.42 (q, 1H, CH, *J* = 8 Hz), 7.07 (d, 1H, furan H, *J* = 4 Hz), 7.18 (d, 1H, furan H, *J* = 4 Hz), 7.15 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.28 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.99 (d, 2H, 4-nitrophenyl), 8.35 (d, 1H, 4-nitrophenyl, *J* = 8 Hz). LC MS: *m/z* = 474 (M+1)

6-[5-(4-methoxyphenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b] [1,3,4]-thiadiazoles (7h)

IR (KBr, γ_{\max} , cm⁻¹): 3085 (ArC-H), 2954 (C-H), 1620 (C=N), 1532 (C=C), ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.84 (d, 6H, CH₃, *J* = 8 Hz), 1.66 (d, 3H, CH₃, *J* = 8 Hz), 1.80-1.85 (m, 1H, CH), 2.39 (s, 3H, Ar-CH₃), 2.42 (d, 2H, CH₂, *J* = 8 Hz), 4.40 (q, 1H, CH, *J* = 8.0 Hz), 6.90 (d, 1H, furan H, *J* = 3.6 Hz), 6.96 (d, 2H, 4-methoxy phenyl), 7.03 (d, 1H, furan H, *J* = 3.6 Hz), 7.12 (d, 2H, ibuprofen, *J* = 8.0 Hz), 7.24 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.19 (d, 2H, 4-methylphenyl, *J* = 8 Hz), 7.36 (d, 2H, 4-methoxyphenyl, *J* = 8 Hz). LC MS: *m/z* = 459 (M+1)

6-[5-(2,4-dichlorophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-b] [1,3,4]-thiadiazoles (7i)

IR (KBr, γ_{\max} , cm⁻¹): 3116 (ArC-H), 2953 (C-H), 1686 (C=N), 1599 (C=C), 850 (C-Cl). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.81 (d, 6H, CH₃, *J* = 8 Hz), 1.72 (d, 3H, CH₃, *J* = 8 Hz), 1.75-1.80 (m, 1H, CH), 2.37 (d, 2H, CH₂, *J* = 8 Hz), 4.25 (q, 1H, CH, *J* = 8 Hz), 6.83 (d, 1H, furan H, *J* = 3.6 Hz), 6.96 (d, 1H, furan H, *J* = 3.6 Hz), 7.02 (d, 2H, 4-isobutylphenyl, *J* = 8 Hz), 7.24 (d, 2H, isobutylphenyl, *J* = 8 Hz), 7.44-7.51 (m, 2H, 2,4-chlorophenyl, *J* = 8 Hz), 7.62 (d, 1H, 2,4-chlorophenyl, *J* = 4 Hz). LC MS: *m/z* (%) = 498 (M+1), 500 (M+3), 502 (M+5)

6-[5-(2-Nitro-4-methoxyphenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl][1,2,4]triazole [3,4-b][1,3,4]-thiadiazoles (7j)

IR (KBr, γ_{\max} , cm⁻¹): 3086 (ArC-H), 2985 (C-H), 1602 (C=N), 1522 (NO₂), 1315 (NO₂). ¹H NMR (400 MHz, CDCl₃, δ ppm): 0.88 (d, 6H, CH₃, *J* = 6.4 Hz), 1.76 (d, 3H, CH₃, *J* = 7.2 Hz), 1.80-1.87 (m, 1H, CH), 2.44 (d, 2H, CH₂, *J* = 7.2 Hz), 4.30-4.35 (q, 1H, CH, *J* = 7.2 Hz), 6.87 (d, 1H, furan H, *J* = 3.6 Hz), 7.00 (d, 1H, furan H, *J* = 3.6 Hz), 7.14

(d, 2H, isobutylphenyl, $J = 8.0$ Hz), 7.16 (d, 2H, isobutylphenyl, $J = 8.0$ Hz), 7.41-7.63 (m, 3H, 2-nitro-4-methoxyphenyl). LC MS (m/z): 503 (M^+)

6-[5-(4-chloro-2-nitrophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl][1,2,4]triazole [3,4-b][1,3,4]-thiadiazoles (**7k**)
IR (KBr, γ_{\max} , cm^{-1}): 3088 (ArC-H), 2989 (C-H), 1600 (C=N), 1540 (NO_2 asymmetric), 1339 (NO_2 symmetric), 885 (C-Cl). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 0.86 (d, 6H, CH_3 , $J = 6.4$ Hz), 1.74 (d, 3H, CH_3 , $J = 7.2$ Hz), 1.82-1.87 (m, 1H, CH), 2.45 (d, 2H, CH_2 , $J = 7.2$ Hz), 4.31-4.36 (q, 1H, CH, $J = 7.2$ Hz), 6.90 (d, 1H, furan H, $J = 3.6$ Hz), 7.08 (d, 1H, furan H, $J = 3.6$ Hz), 7.12 (d, 2H, isobutylphenyl, $J = 8.0$ Hz), 7.15 (d, 2H, isobutylphenyl, $J = 8.0$ Hz), 7.69 (d, 1H, $J=2\text{Hz}$, 4-chloro-2-nitrophenyl), 7.78 (d, 1H, $J=8\text{Hz}$, 4-chloro-2-nitrophenyl), 7.87 (d, 1H, $J=2\text{Hz}$, 4-chloro-2-nitrophenyl). LC MS (m/z): 509 ($M+1$), 511 ($M+3$)

6-[5-(2,4,5-trichloro-2-nitrophenyl)-2-furyl]-3-[1-(4-isobutylphenyl)ethyl][1,2,4] triazole [3,4-b][1,3,4]-thiadiazoles (**7l**)
IR (KBr, γ_{\max} , cm^{-1}): 3090 (ArC-H), 2992 (C-H), 1604 (C=N), 878 (C-Cl). ^1H NMR (400 MHz, CDCl_3 , δ ppm): 0.88 (d, 6H, CH_3 , $J = 6.4$ Hz), 1.77 (d, 3H, CH_3 , $J = 7.2$ Hz), 1.83-1.88 (m, 1H, CH), 2.46 (d, 2H, CH_2 , $J = 7.2$ Hz), 4.33-4.38 (q, 1H, CH, $J = 7.2$ Hz), 6.80 (d, 1H, furan H, $J = 3.6$ Hz), 7.06 (d, 1H, furan H, $J = 3.6$ Hz), 7.11 (d, 2H, isobutylphenyl, $J = 8.0$ Hz), 7.14 (d, 2H, isobutylphenyl, $J = 8.0$ Hz), 8.02 (s, 1H, 2,4,5-trichloro), 8.15 (s, 1H, 2,4,5-trichloro). LC MS (m/z): 532 ($M+1$).

***In vitro* antibacterial assay**

Compounds were also screened for their *in-vitro* antibacterial screening by employing serial plate dilution method [32] at 1.56 to 25 $\mu\text{g}/\text{mL}$ concentrations. Serial dilutions of the drug in Muller Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37° C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37° C for an hour. Using a punch, wells were made on these seeds agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labelled well. A control was also prepared for the plates in the same way using DMSO as a solvent. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [33]. Zone of inhibition and MIC was determined for **7(a-l)** at the concentration range of 1.56-25 $\mu\text{g}/\text{mL}$.

***In vivo* anti-inflammatory assay**

In vivo anti-inflammatory activity of the newly synthesized compounds was determined by carrageen induced paw edema method [34] and the activity was compared with the standard drug Diclofenac Sodium. Wister albino rats of either sex weighing 180-250 g were used for the experiment. They were housed in the clean polypropylene cages and kept under room temperature (25° C), relative humidity (60-70%) in 12 h of light-dark cycle. The animals were given standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during experimental hours. The animals were divided into 18 groups with each group containing 6 animals. A mark was made on the hind paw (left) just below the tibia-tarsal junction, so that every time the paw was dipped in the mercury column up to fixed mark to ensure constant paw volume. The initial paw volume of each rat was noted by plethysmometrically. First group received 0.6% Na CMC and the second group received Diclofenac Sodium at a dose of 10 mg/kg body weight p. o. The 3rd to 18th groups were administered with the test compounds at a dose 10 mg/kg (suspended in 0.6% CMC given p. o.). 30 min after the treatment of test compounds, 0.1 ml of 1% (w/v) carrageenan was injected in the subplantar region of the left hind paw. The right paw served as a reference to non-inflamed paw for comparison. The initial paw volume was measured within 30 s of the injection. The relative increase in paw volume was measured in control, standard and test compounds at 3 h after the carrageenan injection. The difference between the two readings was taken as the volume of oedema, the percentage inhibition by the drugs was calculated using the formula,

$$\text{Percentage of oedema inhibition} = 100 - [(V_{\text{test}}/V_{\text{control}}) * 100];$$

Where, V_{control} = volume of paw oedema in control group;

V_{test} = volume of paw oedema in the test compounds in treated group.

The results were expressed as % inhibition of oedema over the untreated control group.

RESULTS AND DISCUSSION

Chemistry

The synthetic route for the title compounds is depicted in **Scheme 1**. Arylfuroic acids (**6**) were prepared from substituted anilines and furoic acid by Meerwein reaction. Ibuprofen [2-(4-isobutylphenyl) propanoic acid] (**1**) was subjected to esterification followed by hydrazinolysis using hydrazine hydrate to afford 4-isobutylphenylethylhydrazide (**3**). The resulting hydrazide was treated with carbon disulfide and potassium hydroxide to afford the corresponding potassium dithiocarbazinate (**4**) which on reaction with hydrazine hydrate underwent cyclocondensation reaction to yield 4-amino-5-[1-(4-isobutylphenyl)ethyl]-3-mercapto-[1,2,4]-triazole (**5**). Condensation of the above triazole with various substituted arylfuroic acids (**6**) in the presence of phosphorus oxychloride afforded a series of 6-(5-aryl-2-furyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazoles (**7a-l**). Characterization data of **7(a-l)** is given in **Table 1**.

Scheme 1: Synthesis strategy of 6-(5-Aryl-2-furyl)-3-[1-(4-isobutylphenyl)ethyl]-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazoles 7(a-l)

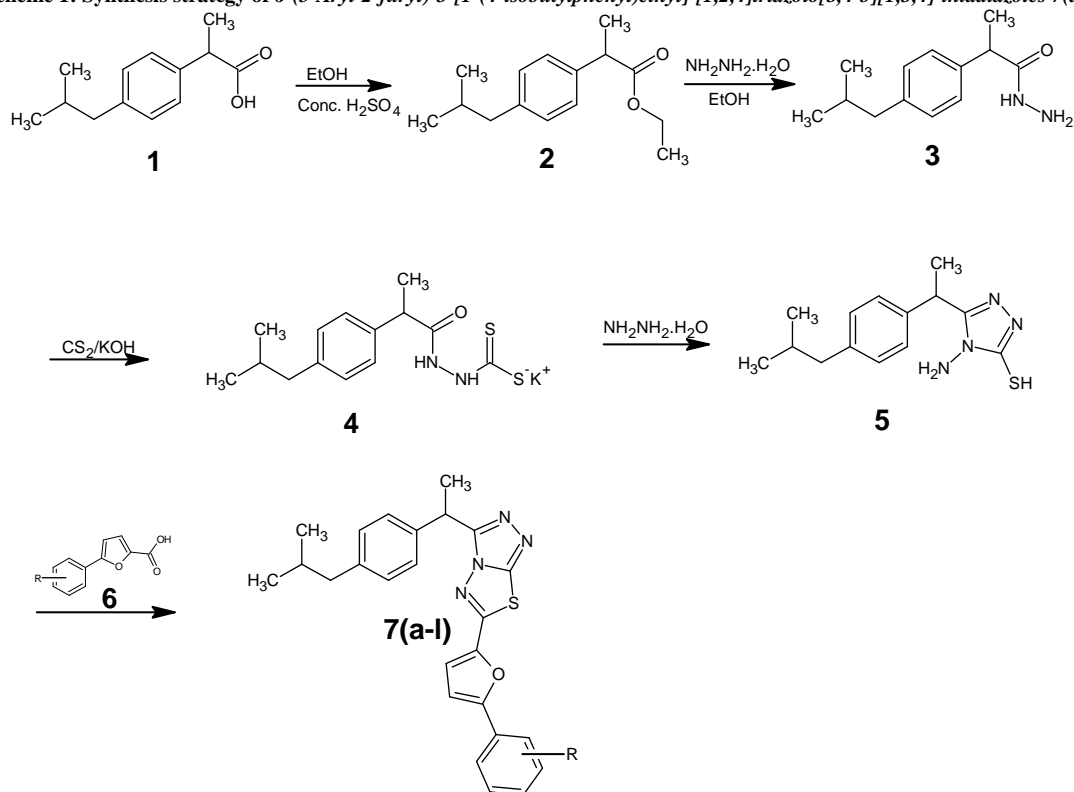


Table 1: Characterization table of triazolothiadiazoles 7(a-l)

Comp.	R	Mol. Formula (Mol. Weight)	M.p. (°C)	Yield (%)	% Analysis of C, H, N Found (Cald.)		
					C	H	N
7a	2-Cl	C ₂₅ H ₂₃ ClN ₄ OS (462.12)	208-210	85	64.84 (64.85)	5.02 (5.01)	12.09 (12.10)
7b	4-Cl	C ₂₅ H ₂₃ ClN ₄ OS (462.12)	215-217	82	64.83 (64.85)	5.05 (5.01)	12.08 (12.10)
7c	4-Br	C ₂₅ H ₂₃ BrN ₄ OS (506.4)	145-146	78	59.15 (59.17)	4.59 (4.57)	11.06 (11.04)
7d	4-CH ₃	C ₂₆ H ₂₆ N ₄ OS (442.57)	162-164	86	70.59 (70.56)	5.95 (5.92)	12.68 (12.66)
7e	2-CF ₃	C ₂₆ H ₂₃ F ₃ N ₄ OS (496.54)	236-238	78	62.87 (62.89)	4.69 (4.67)	11.30 (11.28)
7f	2-NO ₂	C ₂₅ H ₂₃ N ₅ O ₃ S (473.54)	230-232	78	63.44 (63.41)	4.93 (4.90)	14.81 (14.79)
7g	4-NO ₂	C ₂₅ H ₂₃ N ₅ O ₃ S (473.54)	242-244	76	63.40 (63.41)	4.91 (4.90)	14.80 (14.79)
7h	4-OCH ₃	C ₂₆ H ₂₆ N ₄ O ₂ S (458.57)	226-227	80	68.12 (68.10)	5.69 (5.71)	12.20 (12.22)
7i	2,4-Cl ₂	C ₂₅ H ₂₂ Cl ₂ N ₄ OS (497.43)	200-202	72	60.34 (60.36)	4.45 (4.46)	11.24 (11.26)
7j	2-NO ₂ -4-OCH ₃	C ₂₆ H ₂₅ N ₅ O ₄ S (503.57)	206-208	74	62.03 (62.01)	4.99 (5.00)	13.89 (13.91)
7k	4-Cl-2-NO ₂	C ₂₅ H ₂₂ ClN ₅ O ₃ S (507.99)	210-212	70	59.10 (59.13)	4.39 (4.37)	13.81 (13.79)
7l	2,4,5-Cl ₃	C ₂₅ H ₂₁ Cl ₃ N ₄ OS (531.88)	242-244	82	56.48 (56.45)	3.99 (3.98)	10.55 (10.53)

The structures of triazole (**5**) and its triazolothiadiazoles (**7a-l**) derivatives were established on the basis of elemental analyses, IR, ¹H NMR, ¹³C NMR and Mass spectral data. The IR spectrum of triazole (**5**) showed the characteristic

NH₂ absorptions at 3331 cm⁻¹ and 3250 cm⁻¹. The absorption band present at 3092 cm⁻¹, 1601 cm⁻¹ are due to ArC-H, C=N stretching vibration of the molecule. The spectrum also showed NH bending vibration at 1520 cm⁻¹. In the 400 MHz ¹H NMR spectrum of compound (**5**), the doublets at δ 0.80 (6H, J = 6.8 Hz) and δ 1.58 (3H, J = 7.6 Hz) indicated the presence of isopropylmethyl and CH₃ groups respectively. A multiplet in the range δ 1.71-1.78, a doublet at δ 2.35 (J = 7.2 Hz) and a quartet at δ 4.24 (J = 4 Hz) confirmed the presence of isopropylmethyne, methylene and methyne protons respectively. The aromatic protons of (**5**) appeared as two doublets at δ 7.00 and δ 7.08 with J = 8 Hz. Two broad singlets at δ 4.45 and δ 12.12 were due to the NH₂ and NH/SH tautomeric protons. The mass spectrum of (**5**) showed an intense protonated molecular ion (M⁺+1) peak at m/z 277 corresponding to its molecular formula C₁₄H₂₀N₄S.

Formations of the triazolothiadiazoles (**7a-l**) were also confirmed by their elemental analyses, IR, ¹H NMR and mass spectral data. In the IR spectrum of triazolothiadiazole **7c**, absorption bands corresponding to NH₂ group were disappeared. But it showed absorption bands at absorption bands at 3160, 3098 (ArC-H), 2966 (C-H) and 1588 (C=N) cm⁻¹. In the 400 MHz ¹H NMR spectrum of compound **7c**, the signals corresponding to the NH₂ and NH/SH tautomeric protons were disappeared. The spectrum showed two distinct doublets at δ 0.89 (J = 8.0 Hz) and δ 1.76 (J = 8.0 Hz) was also observed for its methyl protons. Isopropyl methyne proton was observed as a multiplet in the range δ 1.80-1.85 and the other methyne proton was observed as a quartet at δ 4.32. Methylene protons were resonated as a doublet at δ 2.43 (J = 8.0 Hz). The spectrum showed two β -protons of furan ring residue resonated at δ 6.86 and δ 7.01 for J = 4 Hz. The aromatic protons of ibuprofen appeared as two doublets at δ 7.11 and δ 7.31 with J = 8.4 Hz. The four aromatic protons of bromophenyl moiety appeared as doublets at δ 7.41 and δ 7.66 with J = 8.8 Hz. The LC MS spectrum of **7c** showed the molecular ion and isotopic peaks at m/z = 506 and 508 in conformity with its molecular formula, C₂₅H₂₃N₄SOBr.

PHARMACOLOGY

Antibacterial activity

Results of antibacterial screening study is depicted in **Table 2**. Antibacterial screening data revealed that all the tested compounds **7(a-l)** showed good to moderate activity against both gram positive and gram negative bacterial strains. Compounds **7a** (2-Cl), **7b** (4-Cl), **7e** (2-CF₃), **7f** (2-NO₂), **7g** (4-NO₂) and **7l** (2,4,5-triCl) showed very good activity against all the bacterial strains at MIC value of 6.25 μ g/mL. Remaining compounds exhibited moderate activity towards all the strains. Structure-activity relationship for the observed activity and substitution in the aryl furan ring can be done. It was clear from the result that chloro substitution in aryl furan ring either at 2nd or 4th enhances the activity. With chlorine at 2,5 position activity was diminished whereas 2,4,5- position enhances the activity. From the four chloro substituted derivatives, it can be concluded that chlorine at 4th position is essential for higher activity. When Chloro group in the 4th position was replaced by bulky bromo, activity got reduced. Further electron releasing methyl, methoxy at 4th position does not enhance the activity whereas the electron releasing trifluoromethyl group at 2nd position has enhances the inhibition. Presence electron withdrawing nitro group at 2nd or 4th position increases the activity. But when chlorine and electron releasing methoxy is present at 4th position with nitro at 2nd position activity was reduced. From structure activity relationship result it is clear that, halogens and nitro group are responsible for greater antibacterial activity.

Table 2: Antibacterial activity of the compounds 7(a-l)

Compound	MIC [μ g/ mL] and zone of inhibition (mm) in parentheses			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
7a	6.25(19-23)	6.25(19-24)	6.25(19-24)	6.25(20-24)
7b	6.25(19-24)	6.25(18-22)	6.25(19-24)	6.25(18-22)
7c	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7d	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7e	6.25(19-24)	6.25(19-24)	6.25(19-24)	6.25(19-24)
7f	6.25(19-24)	6.25(19-24)	6.25(20-24)	6.25(19-24)
7g	6.25(20-24)	6.25(19-24)	6.25(19-24)	6.25(19-24)
7h	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7i	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7j	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7k	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7l	6.25(19-24)	6.25(20-24)	6.25(19-24)	6.25(20-24)
Standard (Ampicillin)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

The MIC values were evaluated at concentration range, 1.56-25 μ g/mL

Antifungal activity data of the newly synthesized compounds is depicted in **Table 3**. Antifungal activity result showed that these compounds are moderate activity towards the fungal strains. Compound **7k** with chloro at 4th position and nitro group at 2nd position has showed very good activity against all the fungal strains. Further,

compound **7a**, **7b**, **7f**, **7i**, **7j** and **7l** exhibited moderate activity. From the screening study, it was clear that these fused triazolothiadiazoles are inactive towards the fungal strains.

Table 3: Antifungal activity of the compounds 7(a-l)

Compound	MIC [$\mu\text{g}/\text{mL}$] and zone of inhibition (mm) in parentheses			
	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>P. marneffeii</i>	<i>T. mentagrophytes</i>
7a	12.5(11-15)	12.5(11-16)	12.5(10-15)	12.5(11-15)
7b	12.5(11-15)	12.5(11-16)	12.5(10-15)	12.5(11-15)
7c	25<(10)	25<(10)	25<(10)	25<(10)
7d	25<(10)	25<(10)	25<(10)	25<(10)
7e	25<(10)	25<(10)	25<(10)	25<(10)
7f	12.5(11-15)	12.5(11-15)	12.5(10-15)	12.5(11-15)
7g	25<(10)	25<(10)	25<(10)	25<(10)
7h	25<(10)	25<(10)	25<(10)	25<(10)
7i	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
7j	12.5(10-15)	12.5(11-16)	12.5(11-15)	12.5(10-15)
7k	6.25(18-22)	6.25(16-20)	6.25(16-20)	6.25(16-20)
7l	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
Standard (Itraconazole)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

The MIC values were evaluated at concentration range, 1.56-25 $\mu\text{g}/\text{mL}$

Anti-inflammatory activity

The anti-inflammatory activity of **7(a-l)** was in the range of 40.40%-68.65% inhibition and is given in **Table 4**. The highest inhibition 68.65% was observed for **7(b)** with 4-chloro substitution in the aryl furanring followed by **7h** with 4-methoxy substitution with 67.70% inhibition. Further compound **7i** with 2,5-dichloro and **7l** with 2,4,5-trichloro substitution showed 65.12% and 63.92% of inhibition respectively. From these three chloro derivatives it is observed that as number of chlorine atom increase in the aryl furan ring, anti-inflammatory activity decreases. Compound **7g** with electron withdrawing nitro at 4th position of aryl furan ring showed less degree of inhibition. Further when this group are replaced by 4-Cl, 4-Br, 4-CH₃, 2-NO₂, 2-NO₂-4-OCH₃ and 4-Cl-2-NO₂ in the aryl furan ring the activity was found to be decreased.

Table 4: Anti-inflammatory activity data of compounds 7(a-l)

Compound	Dose(mg/kg body weight,p.o)	Increase in paw volume in ml MEAN \pm SEM)	% Inhibition of paw oedema
7a	10	0.0896 \pm 0.0035	46.68
7b	10	0.0508 \pm 0.0071	68.65
7c	10	0.0899 \pm 0.0027	44.51
7d	10	0.0925 \pm 0.0033	42.91
7e	10	0.0896 \pm 0.0035	46.68
7f	10	0.0896 \pm 0.0035	46.68
7g	10	0.0698 \pm 0.0041	56.90
7h	10	0.0523 \pm 0.0084	67.70
7i	10	0.0565 \pm 0.0020	65.12
7j	10	0.0896 \pm 0.0035	46.68
7k	10	0.0966 \pm 0.0040	40.40
7l	10	0.0585 \pm 0.0021	63.92
Control	0.1 ml/kg	0.1621 \pm 0.0015	-
Standard	10	0.0390 \pm 0.0026	75.93

Diclofenac Na is used as the standard: N=6 in each group.

Analgesic activity

The compounds which have shown good anti-inflammatory activity was screened for analgesic activity **Table 5**. The tested compounds **7b**, **7g**, **7h**, **7i** and **7l** showed analgesic activity ranging from 57.14% to 75.00%, whereas standard drug Diclofenac showed 77.57% inhibition. The compound **7b** with 2-chloro substitution showed highest inhibition (75.0%) among the tested compounds. The compound **7h** having electron releasing methoxy group at 4th position and the compound **7i** having 2,4-dichloro substitution in the aryl furan ring exhibited good activity (72.68% and 71.96%). On the other hand, compound **7l** with three chlorine atoms showed lesser inhibition than the compound with two chlorine atoms. The introduction of one more chlorine at 5th position has reduced the activity of **7l**. Further replacement of electron withdrawing nitro group at 4th position in place of the methoxy group of **7h** has drastically decreased the analgesic activity.

Table 5: Analgesic activity data of the compounds 7b, 7g, 7h, 7i and 7l

Compound	Before treatment \pm SEM	After treatment \pm SEM (1.5 hours)	% Increase in reaction timeSEM ^a
7b	2.12 \pm 0.53	3.71 \pm 0.52	75.00 ^b
7g	2.24 \pm 0.45	3.52 \pm 0.18	57.14 ^c
7h	2.16 \pm 0.22	3.73 \pm 0.54	72.68 ^c
7i	2.14 \pm 0.44	3.68 \pm 0.41	71.96 ^c
7l	2.29 \pm 0.14	3.65 \pm 0.05	59.38 ^c
Diclofenac	2.14 \pm 0.33	3.80 \pm 0.24	77.57 ^c

^a Results are expressed as their mean values (n=6).

^b P < 0.05, ^c P < 0.01; significant from the control

CONCLUSION

The present study describes an efficient synthesis of fused triazolo-thiadiazole systems starting from well-known anti-inflammatory drug ibuprofen and their antibacterial/anti-inflammatory properties. The results of antibacterial screening studies revealed that compounds containing -Cl, CF₃ and NO₂ groups in the aryl furan ring exhibited higher activity than other compounds. Anti-inflammatory screening study revealed that a compound containing 4-chloro and 4-methoxy substituents in the aryl furan ring are more potent in the series. Further, structure activity study of these compounds suggested that with presence chlorine and electron releasing methoxy groups at 4th position are responsible for better anti-inflammatory and analgesic activity and when these groups was replaced by withdrawing nitro group activity drifted very much. Furthermore, increase in number of chlorine atom anti-inflammatory and analgesic activity got reduced and the activity of these halogenated compounds follows the order 4-Cl > 2,5-Cl₂ > 2,4,5-Cl₃.

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