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Synthesis and anti-inflammatory properties of novel 1,2,4-triazole derivatives

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Abstract This research is based on the pharmacological activity of the triazole ring. In the last decade much work has been conducted on the triazole ring. Scientists have developed many new compounds based on this structure and screened them to obtain molecules with good pharmacological activity. In this research starting from 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione, a series of new 1,2,4-triazole derivatives were prepared. Results revealed that two compounds had anti-inflammatory potency after 4 h greater than that of indomethacin whereas another derivative had less potency than indomethacin.

Keywords Triazole derivatives · Anti-inflammatory agents

Introduction

Derivatives of 1,2,4-triazole have been reported to have interesting biological activity; for example, 5-(2-, 3-, and 4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol derivatives [1], 5-substituted-3-pyridine-1,2,4-triazoles [2], and others [3–22] have anti-inflammatory activity. Triazole, thiadiazole, and triazine derivatives of Indomethacin have been synthesized and tested for anti-inflammatory activity. The test compounds inhibited the induction of gastric mucosal lesions and their protective effects may be

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related to inhibition of lipid peroxidation in gastric mucosa [10]. Moreover, triazoles fused with other heterocyclic rings have attracted particular attention, because of their diverse applications, for example antibacterial, antidepressant, antiviral, antitumor, anti-inflammatory, pesticide, herbicide, and lubricant activity, and as analytical reagents [23, 24]. 1,2,4-Triazoles have been shown to have multifaceted pharmacological properties, including antihypertensive, cardiac stimulant, antimalarial, antifungal, anti-HBV, antimicrobial, anti-cancer, and herbicidal activity [25–34]. Prompted by these findings, it seemed of interest to synthesize new derivatives of triazole and investigate their anti-inflammatory activity to study the effect of positional substitution on biological activity.

Experimental

Chemistry

All melting points are uncorrected and measured by use of an electrothermal capillary melting point apparatus. Infrared spectra were acquired with a Jasco FT/IR-6100 using KBr discs. ^{1}H NMR spectra were acquired with Jeol 270 MHz and Jeol sx 500 MHz spectrometers, using TMS as internal standard. Mass spectra were acquired with a Jeol JMS-AX 500. All reactions were followed and checked by TLC (aluminium-backed plates) with chloroform–methanol 9:1 (*v*/*v*) as mobile phase. For detection the plates were sprayed with iodine.

General procedure for preparation of compound ***2***

Iodomethane (0.25 g, 0.34 ml, 1.728 mmol) was added dropwise and with stirring to a suspension of **1** [35] (1.728 mmol) in absolute ethanol (40 mL). The reaction mixture was boiled with stirring for 3 h. The solvent was removed with a rotary evaporator; the solid product was treated, with stirring, with 10 % KOH (15 mL). The solid product **2** was isolated by filtration, washed with water, dried, and recrystallized.

*3-(4-Chlorophenyl)-5-(methylsulfanyl)-4H-1,2,4-triazol-4-amine (**2**)*

Crystallized from acetic acid, yellow crystals, m.p. 201 °C, yield 70 %. Analysis: for $C_9H_9ClN_4S$, M.Wt. 240.71, calcd: C, 44.91; H, 3.77; N, 23.28. Found: C, 44.54; H, 3.40; N, 23.01. IR (KBr, cm^{-1}): 1,033–1,345 (C–N), 1,430–1,614 (C=C) and 3,199, 3,389 (Prim NH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.57 (3H, *s*, SCH_3), 6.1 (2H, *s*, NH_2, exchangeable with D_2O) and 7.4–7.9 (4H, *m*, aromatic). MS: (*m*/*z*) ~240 (40.65 %).

General procedure for preparation of compound ***3***

A mixture of formaldehyde (40 %, 1.5 mL), *p*-chloroacetophenone (0.001 mol), and compound **1** [35] (0.001 mol) in ethanol was stirred at room temperature for

6 h. The precipitated solid **3** was isolated by suction filtration, washed with ethanol, and recrystallized.

*3-[4-Amino-3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-1-(4-chlorophenyl)propan-1-one (**3**)*

Crystallized from dimethylformamide, white crystals, m.p. 263 °C, yield 70 %. Analysis: for $C_{17}H_{14}Cl_2N_4OS$, M.Wt. 393.29, calcd: C, 51.92; H, 3.59; N, 14.25. Found: C, 51.75; H, 3.39; N, 14.08. IR (KBr, cm^{-1}): 1,123−1,384 (C–N), 1,484–1,634 (C=C), 1,670 (C=O) and 2,922, 3,409 (Prim NH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.1 (2H, *t*, CH_2), 3.7 (2H, *t*, CH_2), 5.9 (2H, *s*, NH_2, exchangeable with D_2O) and 7.2–8 (8H, *m*, aromatic). MS: (*m/z*) $[M + 1]^+$ *m/z* ∼209 (100 %) for ($C_8H_5ClN_3S^{\bullet}$) and $[M]^+$ *m/z* ∼281 (1.12 %) for ($C_{11}H_{10}ClN_4OS^{\bullet}$).

*General procedure for preparation of compound **4***

Absolute triethylamine (0.35 g, 0.48 mL, 3.5 mmol) was added to a suspension of compound **1** under reflux [35] (3.5 mmol) in absolute acetonitrile (70 mL). The clear solution obtained was treated with acetyl chloride (0.273 g, 0.25 mL, 3.5 mmol) and the mixture was heated under reflux for 2.5 h. After cooling, the precipitated product **4** was isolated by filtration, washed with methanol and ether, dried, and recrystallized.

*1-[4-Amino-3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]ethanone (**4**)*

Crystallized from acetic acid, brown crystals, m.p. 125 °C, yield 75 %. Analysis: $C_{10}H_9ClN_4OS$, M.Wt. 268.72, calcd: C, 44.70; H, 3.38; N, 20.85. Found: C, 44.55; H, 3.26; N, 20.64. IR (KBr, cm^{-1}): 1,021–1,322 (C–N), 1,445–1,609 (C=C), 1,689 (C=O), and 3,233, 3,455 (Prim NH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.3 (3H, *s*, CH_3), 6.1 (2H, *s*, NH_2, exchangeable with D_2O) and 7.4–7.9 (4H, *m*, aromatic). MS: (*m/z*) ∼268 (15 %).

*General procedure for preparation of compounds **5a**, **b***

To a stirred suspension of compound **1** [35] (2 mmol), and triethylamine (0.28 mL, 2 mmol) in tetrahydrofuran (20 mL), the appropriate acid chloride, i.e. acetyl chloride or benzoyl chloride (2 mmol), was added. The reaction mixture was stirred at ambient temperature for 12 h. The product **5a**, **b** was isolated by filtration, washed with diethyl ether, and dried. Crystallization was performed from an appropriate solvent.

*N-[3-(4-chlorophenyl)-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl]acetamide (**5a**)*

Crystallized from benzene, dark white crystals, m.p. 231 °C, yield 60 %. Analysis: for $C_{10}H_9ClN_4OS$, M.Wt. 268.72, calcd: C, 44.70; H, 3.38; N, 20.85 Found: C, 44.54; H, 3.16; N, 20.64. IR (KBr, cm^{-1}): 1,017–1,365 (C–N), 1,447–1,601 (C=C),

1,702 (C=O) and 3,300 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.1 (3H, *s*, CH_3), 7.1–7.6 (4H, *m*, aromatic), 8.5 (1H, *s*, NH, exchangeable with D_2O) and 9 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ~268 (23 %).

*N-[3-(4-chlorophenyl)-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl]benzamide (**5b**)*

Crystallized from benzene, white crystals, m.p. 175 °C, yield 65 %. Analysis: for $C_{15}H_{11}ClN_4OS$, M.Wt. 330.79, calcd: C, 54.46; H, 3.35; N, 16.94. Found: C, 54.35; H, 3.25; N, 16.75. IR (KBr, cm^{-1}): 1,067–1,325 (C–N), 1,455–1,601 (C=C), 1,695 (C=O) and 2,922 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 7–8.1 (9H, *m*, aromatic), 9.2 (1H, *s*, NH, exchangeable with D_2O) and 10.3 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ~330 (40 %).

*General procedure for preparation of compound **6***

A solution of bromine (0.48 g, 0.15 mL, 3 mmol) in absolute methanol (10 mL) was added dropwise with stirring to a solution of compound **1** [35] (3 mmol) in absolute methanol (125 mL) under reflux. The mixture was heated under reflux for 2.5 h and cooled. The precipitated product, **6**, was isolated by filtration, washed with ether, dried, and recrystallized.

*4-(Bromoamino)-5-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**6**)*

Crystallized from dimethylformamide, brown crystals, m.p. 116 °C, yield 65 %. Analysis: for $C_8H_6BrClN_4S$, M.Wt. 305.58, calcd: C, 31.44; H, 1.98; N, 18.33. Found: C, 31.35; H, 1.63; N, 17.98. IR (KBr, cm^{-1}): 1,032–1,344 (C–N), 1,436–1,612 (C=C) and 2,865 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.3 (1H, *s*, NH, exchangeable with D_2O), 7.6–8 (4H, *m*, aromatic) and 9.2 (1H, *s*, NH, exchangeable with D_2O). MS: $[M + 1]^+$ *m*/*z* ~306 (1.62 %).

*General procedure for preparation of compounds **7a**, **b***

A mixture of **1** [35] (0.01 mol) and the corresponding aromatic aldehyde, i.e. 4-methoxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde (0.01 mol) in ethanol (25 mL) was treated with concentrated HCl (0.5 mL) and heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the separated crystalline compound was filtered and recrystallized to give compounds **7a**, **b**.

*5-(4-Chlorophenyl)-4-[(E)-(4-methoxybenzylidene)amino]-4H-1,2,4-triazole-3-thiol (**7a**)*

Crystallized from acetic acid, white crystals, m.p. 241 °C, yield 60 %. Analysis: for $C_{16}H_{13}ClN_4OS$, M.Wt. 344.81, calcd: C, 55.73; H, 3.80; N, 16.25. Found: C, 55.65; H, 3.67; N, 16.04. IR (KBr, cm^{-1}): 1,020–1,410 (C–N) and 1,450–1,600 (C=C).

^{1}H NMR (DMSO-d_6, δ ppm): 3.6 (3H, *s*, OCH_3), 7.2–8.7 (8H, *m*, aromatic), 10 (1H, *s*, CH) and 12.5 (1H, *s*, SH, exchangeable with D_2O). MS: (*m*/*z*) ~344 (13 %).

*4-[(E)-{[3-(4-chlorophenyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]imino}methyl]-2-methoxyphenol (**7b**)*

Crystallized from benzene, brown crystals, m.p. 270 °C, yield 70 %. Analysis: for $C_{16}H_{13}ClN_4O_2S$, M.Wt. 360.81, calcd: C, 53.26; H, 3.63; N, 15.53. Found: C, 53.11; H, 3.54; N, 15.35. IR (KBr, cm^{-1}): 1,051–1,351 (C–N), 1,480–1,610 (C=C) and 3,435 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (3H, *s*, OCH_3), 5.6 (1H, *s*, OH, exchangeable with D_2O), 7.4–8.3 (7H, *m*, aromatic), 9.7 (1H, *s*, CH) and 12.8 (1H, *s*, SH, exchangeable with D_2O). MS: (*m*/*z*) ~360 (5 %).

*General procedure for preparation of compounds **8a**, **b***

Equimolar proportions of **7a**, **b** and chloroacetic acid were dissolved in ethanol containing 3–4 drops pyridine and heated under reflux for 2–3 h. The reaction mixture was poured into cold water and the separated crystalline compound was isolated by filtration and recrystallized to give compounds **8a**, **b**.

*({5-(4-Chlorophenyl)-4-[(E)-(4-methoxybenzylidene)amino]-4H-1,2,4-triazol-3-yl}sulfanyl)acetic acid (**8a**)*

Crystallized from dimethylformamide, yellow crystal, m.p. 162 °C, yield 90 %. Analysis: for $C_{18}H_{15}ClN_4O_3S$, M.Wt. 402.85, calcd: C, 53.67; H, 3.75; N, 13.91. Found: C, 53.55; H, 3.60; N, 13.79. IR (KBr, cm^{-1}): 1,020–1,260 (C–N), 1,427–1,563 (C=C), 1,732 (C=O) and 3,416 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.9 (3H, *s*, OCH_3), 4.3 (2H, *s*, CH_2), 7−8.4 (8H, *m*, aromatic), 9.2 (1H, *s*, CH) and 10.5 (1H, *s*, OH, exchangeable with D_2O). MS: (*m*/*z*) ~402 (6 %).

*({5-(4-Chlorophenyl)-4-[(E)-(4-hydroxy-3-methoxybenzylidene)amino]-4H-1,2,4-triazol-3-yl}sulfanyl)acetic acid (**8b**)*

Crystallized from dimethylformamide, dark yellow crystals, m.p. 87 °C, yield 70 %. Analysis: for $C_{18}H_{15}ClN_4O_4S$, M.Wt. 418.85, calcd: C, 51.62; H, 3.61; N, 13.38. Found: C, 51.53; H, 3.59; N, 13.04. IR (KBr, cm^{-1}): 1,022–1,287 (C–N), 1,431–1,586 (C=C), 1,726 (C=O) and 3,409 (OH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (3H, *s*, OCH_3), 4 (2H, *s*, CH_2), 6.2 (1H, *s*, OH, exchangeable with D_2O), 6.9–7.8 (7H, *m*, aromatic), 8.6 (1H, *s*, CH) and 10.2 (1H, *s*, OH, exchangeable with D_2O). MS: *m*/*z* ~418 (0.52 %).

*General procedure for preparation of compound **9***

Triethylamine (0.15 g, 0.2 mL, 1.443 mmol) was added to a boiling suspension of **1** [35] (1.443 mmol) in absolute ethanol (10 mL). The mixture was boiled for 5 min and then ethyl bromoacetate (0.24 g, 0.16 mL, 1.443 mmol) was added. The

mixture was boiled with stirring for 2 h. The separated product **9** was isolated by filtration and recrystallized.

*Ethyl{[4-amino-5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (**9**)*

Crystallized from benzene, dark white crystals m.p. 132 °C, yield 65 %. Analysis: for $C_{12}H_{13}ClN_4O_2S$, M.Wt. 312.77, calcd: C, 46.08; H, 4.19; N, 17.91. Found: C, 45.91; H, 4.06; N, 17.78. IR (KBr, cm^{-1}): 1,026–1,310 (C–N), 1,451–1,600 (C=C), 1,739 (C=O) and 3,255, 3,425 (Prim NH). ^{1}H NMR (DMSO-d_6, δ ppm): 1.2 (3H, *t*, CH_3), 4.1 (2H, *q*, CH_2), 4.4 (2H, *s*, CH_2), 5.8 (2H, *s*, NH_2, exchangeable with D_2O) and 7.4−8.1 (4H, *m*, aromatic). MS: (*m*/*z*) ∼312 (21 %).

*General procedure for preparation of compound **10***

A solution of compound **9** (10 mmol) in *n*-butanol was heated under reflux with hydrazine hydrate (25 mmol) for 4 h. After cooling it to room temperature, a white solid appeared. The solid was recrystallized from dimethyl sulfoxide–water (1:1) to afford the desired product **10**.

*2-{[4-Amino-5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetohydrazide (**10**)*

Crystallized from dimethyl sulfoxide–water (1:1), white crystals, m.p. 195 °C, yield 60 %. Analysis: for $C_{10}H_{11}ClN_6OS$, M.Wt. 298.75, calcd: C, 40.20; H, 3.71; N, 28.13. Found: C, 40.10; H, 3.66; N, 28.01. IR (KBr, cm^{-1}): 1,006–1,368 (C–N), 1,463–1,619 (C=C), 1,717 (C=O), 3,295 (Sec NH) and 3,330, 3,400 (Prim NH) .^{1}H NMR (DMSO-d_6, δ ppm): 2.43 (2H, *s*, NH_2, exchangeable with D_2O), 3.7 (2H, *s*, CH_2), 5.7 (2H, *s*, NH_2, exchangeable with D_2O), 7.5–8 (4H, *m*, aromatic) and 13 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ∼298 (0.19 %).

*General procedure for preparation of compound **11***

A mixture of compound **9** (1.25 mmol) and sodium methoxide (from 0.03 g, 1.25 mmol, sodium) in methanol (20 mL) was boiled with stirring for 5 h. The reaction mixture was neutralized with dilute HCl (1:1). The precipitate of product **11** was isolated by filtration, washed with ether, dried, and recrystallized.

*3-(4-Chlorophenyl)-5H-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazin-6(7H)-one (**11**)*

Crystallized from acetic acid, white crystals, m.p. >300 °C, yield 90 %. Analysis: for $C_{10}H_7ClN_4OS$, M.Wt. 266.70, calcd: C, 45.03; H, 2.65; N, 21.01. Found: C, 44.95; H, 2.54; N, 20.89. IR (KBr, cm^{-1}): 1,092–1,315 (C–N), 1,465–1,542 (C=C), 1,632 (C=O) and 3,456 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 3.8 (2H, *s*, CH_2), 7.1–7.9 (4H, *m*, aromatic) and 8.9 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ∼266 (32 %).

General procedure for preparation of compound ***12***

A mixture of **1** [35] (0.01 mol), phenacyl bromide, and fused sodium acetate (0.02 mol) in ethanol (40 mL) was heated under reflux for 2 h. The product obtained was collected by filtration, washed with water, and recrystallized from the appropriate solvent to give **12**.

*3-(4-Chlorophenyl)-6-phenyl-5H-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazine (**12**)*

Crystallized from dimethylformamide, brown crystals, m.p. 280 °C, yield 75 %. Analysis: for $C_{16}H_{11}ClN_4S$, M.Wt. 326.80, calcd: C, 58.80; H, 3.39; N, 17.14. Found: C, 58.73; H, 3.25; N, 17.03. IR (KBr, cm^{-1}): 1,016–1,294 (C–N), 1,455–1,600 (C=C) and 3,456 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 5.2 (1H, *s*, CH), 7.1–8.3 (9H, *m*, aromatic) and 9 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ~326 (12 %).

General procedure for preparation of compound ***13***

A mixture of **1** [35] (0.01 mol), chloroacetone (0.01 mol), and fused sodium acetate (0.02 mol) in ethanol (40 mL) was heated under reflux for 2 h. The product obtained was collected by filtration, washed with water, and recrystallized from the appropriate solvent to give **13**.

*3-(4-Chlorophenyl)-6-methyl-5H-[1, 2, 4]triazolo[3,4-b][1, 3, 4]thiadiazine (**13**)*

Crystallized from benzene, dark brown crystals, m.p. 130 °C, yield 85 %. Analysis: for $C_{11}H_9ClN_4S$, M.Wt. 264.73, calcd: C, 49.91; H, 3.43; N, 21.16. Found: C, 49.89; H, 3.14; N, 20.94. IR (KBr, cm^{-1}): 1,037–1,321 (C–N), 1,464–1,633 (C=C) and 3,386 (Sec NH). ^{1}H NMR (DMSO-d_6, δ ppm): 2.3 (3H, *s*, CH_3), 5 (1H, *s*, CH), 7.1−7.6 (4H, *m*, aromatic) and 8.5 (1H, *s*, NH, exchangeable with D_2O). MS: (*m*/*z*) ~264 (3 %).

Anti-inflammatory activity

The anti-inflammatory testing was performed in accordance with the method of Winter et al. [36]. Paw edema was induced in rats by subcutaneous (s.c.) injection of 0.1 mL 1 % (*w*/*v*) carrageenan in distilled water in the sub-plantar region of their left hind paws. A group of rats was left without any treatment but given the same volume of the solvent (distilled water) and kept as controls. The selected compounds were administered at doses of (25 mg/kg, p.o.). Indomethacin (20 mg/kg, p.o.) was used as reference drug. The paw volumes of the rats were measured, by use of a plethysmometer, before injection of 1 % carrageenan and after different times (1, 2, 3, and 4 h). Edema and inhibition rates for each group were calculated after the above-mentioned times as follows:

$$\text{Edema } (\%) = [Vt - Vo/Vo] \times 100$$

$$\text{Inhibition } (\%) = [Ec - Et/Ec] \times 100$$

where, *Vo* is the volume before carrageenan injection (mL), *Vt* is the volume *t* hours after carrageenan injection (mL), *E*c is the incidence of edema in the control group, and *E*t is the incidence of edema of the treated group.

Animals

The rats used in this study were procured from the Animal House Colony at the National Research Centre (NRC), Egypt. Wistar rats of both sexes, 150–200 g b.wt. were used. All animals were housed under standard conditions of natural 12 h light and dark cycle with free access to food and water. Animals were allowed to adapt to the laboratory environment for one week before experimentation. All animal procedures were performed after approval from the Ethics Committee of The National Research Centre, Egypt, and in accordance with the recommendations of the proper care and use of laboratory animals.

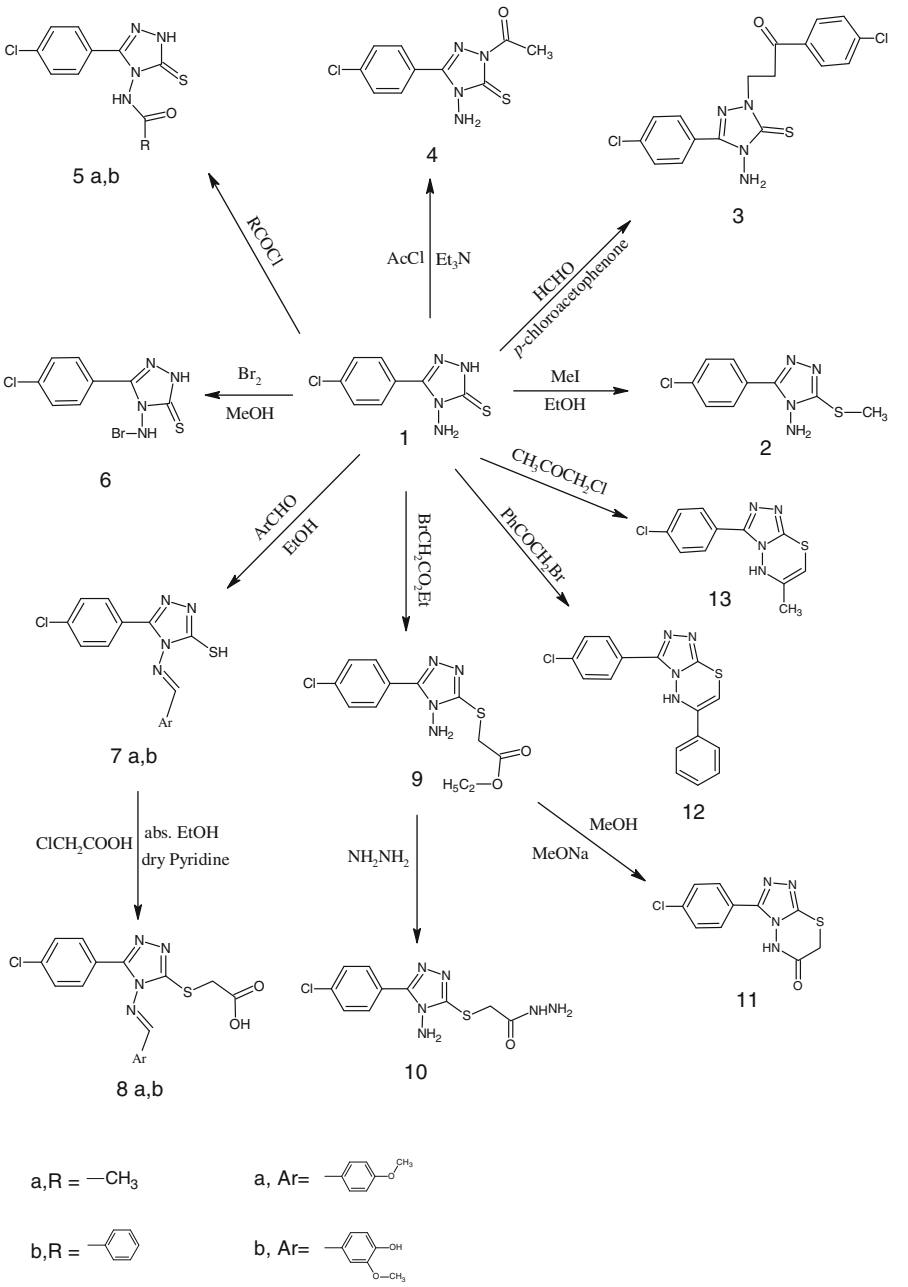
Statistical analysis

Statistical analysis of results was performed by use of SPSS statistics software 17.0 (Released Aug. 23, 2008), Chicago, USA.

Results and discussion

Chemistry

A new 3-(4-chlorophenyl)-5-(methylsulfanyl)-4*H*-1,2,4-triazol-4-amine **2** was synthesized by reaction of 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **1** [35] with methyl iodide. Compound **1** [35] was also reacted with *p*-chloroacetophenone and formaldehyde to give 3-[4-amino-3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]-1-(4-chlorophenyl)propan-1-one **3**. Compound **1** [35] was also reacted with acetyl chloride to give 1-[4-amino-3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]ethanone **4**. Compound **1** [35] was also reacted with appropriate acid chlorides to give *N*-[3-(4-chlorophenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]substituted compounds **5a**, **b**. Compound **1** [35] was also reacted with bromine to give 4-(bromoamino)-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **6**. Compound **1** [35] was also reacted with various aromatic aldehydes to give 5-(4-chlorophenyl)-4-[(*E*)-(substituted)amino]-4*H*-1,2,4-triazole-3-thiols **7a**, **b** which were then reacted with chloroacetic acid to give 5-(4-chlorophenyl)-4-[(*E*)-(substituted) amino]-4*H*-1,2,4-triazol-3-yl}sulfanyl) acetic acids **8a**, **b**. Compound **1** [35] was also reacted with ethyl bromoacetate to give ethyl{[4-amino-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl] sulfanyl}acetate **9** which was then reacted with hydrazine hydrate and with sodium methoxide to give



Scheme 1 Preparation of a series of new 1,2,4-triazole derivatives from 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione

Table 1 Anti-inflammatory activity of selected compounds (**2**, **3**, **5a**, **5b**, **6**, **8a**, **8b**, **9**, **10**, and **13**) against carrageenan-induced paw edema in rats ($n = 6$)

Compound	1 h			2 h			3 h			4 h		
	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)
Control	48.2 ± 3.76[b]	0	–	51.6 ± 4.37[b]	0	–	53.0 ± 3.63[b]	0	–	54.5 ± 2.42[b]	0	–
Indomethacin	27.7 ± 1.75[a]	42.58	100	29.8 ± 2.48[a]	42.23	100	30.9 ± 2.43[a]	41.62	100	30.9 ± 3.02[a]	43.40	100
2	28.1 ± 1.56[a]	41.73	98.0	29.2 ± 2.32[a]	43.41	102.8	30.4 ± 2.98[a]	42.53	102.2	31.1 ± 2.87[a]	42.99	99.1
3	17.1 ± 1.35[ab]	64.52	151.5	18.8 ± 1.82[ab]	63.64	150.7	20.8 ± 1.79[a]	60.79	146.1	20.9 ± 1.98[ab]	61.68	188.2
5a	29.0 ± 1.73[a]	39.90	93.7	31.4 ± 3.01[a]	39.25	92.9	36.3 ± 3.44[a]	31.48	75.6	37.2 ± 2.74[a]	31.71	73.1
5b	24.3 ± 1.86[a]	49.52	116.3	29.2 ± 2.09[a]	43.38	102.7	29.4 ± 1.60[a]	44.46	106.8	33.4 ± 2.10[a]	38.69	89.2
6	26.8 ± 2.62[a]	44.48	104.5	29.6 ± 1.93[a]	42.57	100.8	31.8 ± 2.43[a]	39.94	95.9	31.4 ± 1.64[a]	42.30	97.5
8a	37.3 ± 1.63[ab]	22.64	53.2	36.7 ± 3.71[a]	28.83	68.3	41.8 ± 1.61[ab]	21.06	50.6	43.3 ± 1.20[ab]	20.64	47.6
8b	20.5 ± 1.84[a]	57.39	134.8	24.3 ± 2.07[a]	52.95	125.4	27.3 ± 2.76[a]	48.41	116.3	32.4 ± 1.57[a]	40.52	93.4
9	25.8 ± 1.20[a]	46.41	108.9	26.8 ± 0.95[a]	48.11	113.9	31.4 ± 2.76[a]	40.68	97.7	33.3 ± 2.59[a]	38.92	89.7
10	22.7 ± 2.32[a]	52.95	124.4	25.4 ± 2.26[a]	50.89	120.5	26.9 ± 2.57[a]	49.18	118.2	28.1 ± 2.93[a]	48.52	111.8
13	30.7 ± 3.05[a]	36.32	85.3	32.6 ± 2.49[a]	36.83	87.2	37.5 ± 2.71[a]	29.15	70.0	38.0 ± 2.04[a]	30.20	69.6

[a] $P < 0.05$: Statistically significantly different from control (Dunnett's test)

[b] $P < 0.05$: Statistically significantly different from indomethacin (Dunnett's test)

2-{[4-amino-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl}aceto hydrazide **10** and 3-(4-Chlorophenyl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6 (7*H*)-one **11**, respectively. Compound **1** [35] also reacted with phenacyl bromide and with chloroacetone to give 3-(4-chlorophenyl)-6-phenyl-5*H*-[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazine **12** and 3-(4-chlorophenyl)-6-methyl-5*H*-[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazine **13**, respectively (Scheme 1).

Biological activity

Some of the synthesized compounds were evaluated for possible anti-inflammatory effects in a rat model of carrageenan-induced paw edema. Carrageenan-induced paw edema is a widely used animal model for determining the acute phase of inflammation. (Table 1) shows the effect of selected compounds (**2**, **3**, **5a**, **5b**, **6**, **8a**, **8b**, **9**, **10**, and **13**) on carrageenan-induced paw edema in rats in comparison with indomethacin as reference drug. Intra-plantar injection of carrageenan in rats led to an increase in paw volume denoting edema in the control non-treated group, as shown in Table 1. It was noticed that all the synthesized derivatives in oral doses of 25 mg/kg significantly reduced paw edema throughout the 4-h time period in comparison with the control non-treated group. The anti-inflammatory potency of compounds **2**, **3**, **5a**, **5b**, **6**, **8a**, **8b**, **9**, **10**, and **13** was calculated by comparing their inhibition after different times with that for animals receiving indomethacin, as standard anti-inflammatory drug. The anti-inflammatory potency of the tested compounds after 4 h, compared with indomethacin, were, in descending order, 188.2, 111.8, 99.1, 97.5, 93.4, 89.7, 89.2, 73.1, 69.6, and 47.6 % for **3**, **10**, **2**, **6**, **8b**, **9**, **5b**, **5a**, **13**, and **8a**, respectively. Administration of indomethacin significantly reduced carrageenan-induced edema starting from the first hour and was persistent until the end of the experiment. The inhibitory effect of indomethacin on paw edema was 42.58, 42.23, 41.62, and 43.40 % at the 1st, 2nd, 3rd and 4th hour, respectively. It is noteworthy to mention that derivatives **3** and **10** had anti-inflammatory potency after 4 h greater than that of indomethacin. Derivative **8a** had the least potency. Analysis of structure–anti-inflammatory activity relationships for the tested derivatives revealed that the triazole N-2 and S substituents greatly affect anti-inflammatory activity. Compound **10** is more active than compound **9**, possibly as a result of replacing the OC_2H_5 group by $NHNH_2$. Also, compound **5b** is more active than compound **5a**, possibly because of presence of the benzene ring in **5b** instead of the methyl group in **5a**.

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