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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anti-inflammatory activity of some pyrazole derivatives

Samir M. El-Moghazy · Flora F. Barsoum · Hamdy M. Abdel-Rahman · Adel A. Marzouk

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Abstract A novel series of pyrazoles containing benzenesulfonamides, 1,3,4-oxadiazole-2-thiones, 4-substituted-1,2,4-triazole-3-thiones, and 2-substituted-1,3,4-thiadiazoles has been synthesized. Anti-inflammatory activity of some synthesized compounds was evaluated in vivo utilizing a standard acute carrageenan-induced paw edema method. The most active anti-inflammatory agents **3**, **8f**, and **10f** were evaluated for ulcerogenic liability in rats compared to indomethacin and celecoxib as reference standards. Molecular modeling studies were initiated herein to validate the attained pharmacological data and provide understandable evidence for the observed anti-inflammatory behavior.

Keywords Anti-inflammatory activity · COX-2 · Pyrazole · Synthesis · Oxadiazoles · 1,2,4-Triazoles

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S. M. El-Moghazy · F. F. Barsoum
Faculty of Pharmacy, Pharmaceutical Chemistry Department, Cairo University, Cairo, Egypt

H. M. Abdel-Rahman (✉)
Faculty of Pharmacy, Medicinal Chemistry Department, Assiut University, Assiut 71526, Egypt
e-mail: hamdym01@yahoo.com

A. A. Marzouk
Faculty of Pharmacy, Pharmaceutical Chemistry Department, Al-Azhar University, Assiut branch, Assiut, Egypt

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of pain and inflammation (Amin *et al*., 2009) by counteracting the cyclooxygenase enzymes (COX). However, long-term therapy may cause gastrointestinal complications ranging from stomach irritation to life-threatening gastrointestinal ulceration and bleeding (Wolfe *et al*., 1999). Therefore, it is important to find new anti-inflammatory drugs with a potential for clinical use and not associated with adverse effects. Pyrazole derivatives represent an important class of heterocycles due to their highly pronounced biological and pharmacological activities. Several pyrazole compounds have been reported to be potential therapeutic agents for the treatment of inflammation (Ranatunge *et al*., 2004; Szabó *et al*., 2008; Bekhit and Abdel-Aziem, 2004; Bekhit *et al*., 2006) including the marketed selective COX-2 drug, celecoxib (Fig. 1) that have been shown to be well tolerated with reduced gastrointestinal side effects (Sakya *et al*., 2008). Moreover, various substituted pyrazoles were reported to possess antitumor properties (Rostom *et al*., 2003; Lin *et al*., 2007), others were used for treating Alzheimer's disease (Gökhan-Kelekçi *et al*., 2007) and acquired immunodeficiency syndrome (AIDS) (Shen *et al*., 2004).

Motivated by the aforementioned findings and as a continuation of our on-going program in the field of anti-inflammatory agents (Bekhit *et al*., 2006; Barsoum *et al*., 2006), we intended to investigate the synthesis of new pyrazoles bridged to other anti-inflammatory nuclei as 1,3,4-oxadiazoles, 1,2,4-triazoles, and 1,3,4-thiadiazoles (Abdel-Rahman and Hussien, 2006; Amir and Kumar, 2005) to develop new anti-inflammatory drugs with greater activity and less side effects.

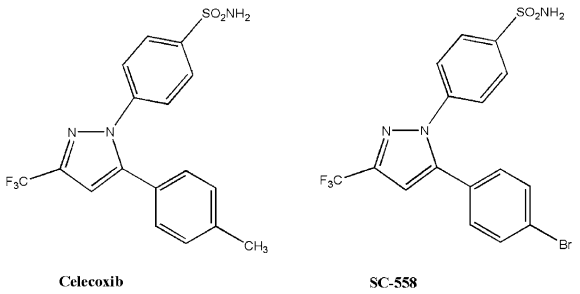


Fig. 1 Structures of the selective COX-2 inhibitors, celecoxib and SC-558

Results and discussion

Chemistry

Reaction of β-diketone **1c** with 4-hydrazino-*N*-(substituted)benzenesulfonamide hydrochlorides in absolute ethanol containing sodium acetate and glacial acetic acid, afforded directly 4-[5-(4-chlorophenyl)-3-methyl-1*H*-pyrazol-1-yl]-*N*-(substituted) benzenesulfonamides **3** and **4**. The IR spectra of the products revealed the absence of a band assignable for the two carbonyl groups, while the ^{1}H-NMR spectra exhibited the pyrazole proton (*H-4*) as a sharp singlet signal at $\delta = 7.0$–7.2 ppm. In addition, the mass spectra exhibited the parent molecular ion peaks confirming the assumed structures.

On the other hand, reaction of **1a–c** with ethylhydrazino acetate hydrochloride followed by refluxing with hydrazine hydrate afforded the acid hydrazide derivatives **6a–c** in good yields (Scheme 2). The IR spectrum of **6c** revealed strong sharp bands at $\nu = 3291$, 3209 cm^{-1} region attributed to the amino and amidic bands and a strong carbonyl stretching vibration band at $\nu = 1665$ cm^{-1}. ^{1}H-NMR spectrum of **6c** exhibited the aminic and amidic protons as singlet signals at $\delta = 4.3$ and 9.3 ppm, respectively (deuterium exchangeable). Moreover, refluxing **6a–c** with CS_2/KOH in ethanol yielded exclusively the 5-(3-methyl-5-substituted-1*H*-pyrazol-1-ylmethyl)-3*H*-1,3,4-oxadiazole-2-thiones **7a–c** in good yields and their structures were confirmed on the basis of their elemental and spectral data (Scheme 2). The IR spectra of the products revealed a strong band at $\nu = 3446$–3422 cm^{-1} region attributed for one amino group and the absence of a band assignable for the carbonyl group at 1665 cm^{-1} region confirming the cyclized form structure. In addition, ^{1}H-NMR spectra displayed only one D_2O exchangeable NH proton as singlet signal at $\delta = 7.5$–7.9 ppm and the mass spectra exhibited the parent molecular ion peaks confirming also the cyclized structure.

Meanwhile, reaction of **6a–c** with isothiocyanates in refluxing ethanol gave directly 2-[2-(3-methyl-5-substituted-1*H*-pyrazol-1-yl)acetyl]-4-substituted thiosemicarbazides **8a–r** (Scheme 3). The structures of **8a–r** were established through spectroscopic (IR, ^{1}H-NMR, and MS) as well as elemental analyses data. ^{1}H-NMR spectra exhibited three singlet signals at $\delta = 7.4$–11.3 region assignable for the amidic protons (disappeared upon deuteration), and the mass spectra exhibited the parent molecular ion peaks confirming the assumed structures. On the other hand, refluxing **8** with sodium hydroxide in ethanol afforded the 1,2,4-triazole-3-thiones derivatives **9**, while stirring **8** with sulfuric acid yielded the 1,3,4-thiadiazole derivatives **10** through intramolecular cyclization onto the carbonyl group (Scheme 3). The IR spectra of **9** and **10** derivatives reveal the absence of a band assignable for the carbonyl group at 1741–1674 cm^{-1} region confirming the cyclized structures. The ^{1}H-NMR spectra of **9** and **10** strongly support the presumed cyclized structures, exhibiting only one D_2O exchangeable amino signal at $\delta = 7.2$–15.2 and at 7.8–11.8 ppm for **9** and **10**, respectively, and the mass spectra exclude any other possible structure, revealing the expected molecular ion peaks.

Anti-inflammatory activity

The anti-inflammatory activity of 13 representative compounds was determined in vivo by the acute carrageenan-induced paw edema standard method in rats (Winter *et al*., 1962). From the obtained results (Table 1), it has been observed that several newly prepared compounds (**3**, **4**, **8f**, and **10r**) revealed better anti-inflammatory properties (61.12–62.67% inhibition of edema) comparable to that of indomethacin (60.8% inhibition of edema). In addition, compound **10f** seems to be the most effective prepared anti-inflammatory agent, revealed better activity (64.93% inhibition of edema) than both indomethacin and celecoxib (reference standards).

However, comparing the activity of 1,3,4-oxadiazole-2-thione compounds **7a–c**, it was observed that replacing the alkyl group by aromatic moiety at position 5, increased slightly the anti-inflammatory activity. In addition, comparing the activity of thiosemicarbazide compounds **8d**, **8e**, and **8f**, it has been noticed that substitution of the phenyl group with an electron-withdrawing group (NO_2) **8f** (62.67% inhibition of edema), seems more favorable for constructing an anti-inflammatory active agent than the case of substitution with an electron-donating groups (CH_3, OCH_3) as exhibited in **8d** and **8e** (40.83 and 46.41% inhibition of edema, respectively). Meanwhile, upon cyclization of the highly potent thiosemicarbazide **8f** using sulfuric acid, the obtained 1,3,4-thiadiazole **10f** (64.93% inhibition of edema) showed a significant increase in the anti-inflammatory activity, while remarkable decrease in the potency was observed upon cyclization of **8f** using

Table 1 Anti-inflammatory activity of the tested compounds using carrageenan-induced paw edema in rats

Compound	Percentage inhibition of edema ± SE		
	1 h	2 h	3 h
Control	0.00	0.00	0.00
Indomethacin	31.51 ± 2.320	40.13 ± 2.911	60.81 ± 3.152
Celecoxib	31.43 ± 2.871	45.75 ± 3.410	63.78 ± 3.301
3	37.21 ± 1.874[b]	49.81 ± 1.192[b]	62.47 ± 1.156[b]
4	34.11 ± 1.449[b]	44.32 ± 3.273[b]	62.03 ± 1.970[b]
7a	14.38 ± 3.095[b]	21.53 ± 3.707[b]	34.88 ± 2.693[b]
7b	26.31 ± 3.031[b]	34.17 ± 2.708[b]	48.71 ± 2.261[b]
7c	15.44 ± 3.028[b]	31.22 ± 3.233[b]	39.18 ± 2.540[b]
8d	12.27 ± 3.572[a]	22.90 ± 4.099[a]	40.83 ± 3.218[a]
8e	30.33 ± 2.957[b]	41.38 ± 3.471[b]	46.41 ± 3.704[b]
8f	36.13 ± 2.191[b]	44.43 ± 2.396[b]	62.67 ± 1.179[b]
9d	13.15 ± 2.591[a]	22.73 ± 4.901[a]	33.42 ± 2.955[a]
9f	16.61 ± 2.925[b]	27.15 ± 2.927[b]	36.38 ± 2.313[b]
10f	37.52 ± 1.031[b]	49.96 ± 2.272[b]	64.93 ± 2.222[b]
10h	26.86 ± 1.556[b]	42.25 ± 1.327[b]	54.11 ± 1.255[b]
10r	32.79 ± 1.477[b]	45.01 ± 1.026[b]	61.12 ± 0.921[b]

All values are represented as means of 6 experiments ± SE. Statistical analysis was carried out by one-way ANOVA test followed by Dunnett's *t* test at $P < 0.05$ and 0.01

[a] Significant difference from the control value at $P < 0.05$

[b] Significant difference from the control value at $P < 0.01$

sodium hydroxide to obtain the 1,2,4-triazole **9f** (36.38% inhibition of edema).

Ulcerogenic liability

Ulcerogenic liability of the most promising prepared anti-inflammatory active agents (**3**, **8f**, and **10f**) was determined following the previously reported standard method (Barsoum *et al*., 2006, 2009) using indomethacin as a reference standard. From the obtained data (Table 2) it has been noticed that all compounds reveal lower ulcer indexes (5.2–18.6) and they are considered more safer than indomethacin itself which reveals ulcer index 20.4.

Table 2 Ulcerogenic liability of the highly active anti-inflammatory compounds

Compound	Number of animals with ulcer	Average severity	Ulcer index
Control	0/5	0.0	0.0
Indomethacin	5/5	2.8	20.4
3	2/5	0.4	5.2
8f	5/5	2.6	18.6
10f	4/5	1.8	15.6

Table 3 The final docking score (*S*) for the first poses of compound **3** in the active site of COX-2

Pose number	The final score (*S*) (kcal/mol)
1	−10.3836
2	−10.2233
3	−9.8776
Average	−10.1615

Molecular modeling studies

Molecular modeling study of a highly observed anti-inflammatory active agent **3** was initiated herein to understand the observed pharmacological data. Docking studies were performed by Molecular Operating Environment (MOE, Version 2008, Chemical Computing Group Inc., Montreal, Quebec, Canada), exhibiting COX-2 enzyme co-crystallized with SC-558 (PDB ID: 1CX2), which used as a template in this study (Kurumbail *et al*., 1996). 100 Docking interactions for each ligand were performed and the top score docking energy value was recorded (Table 3).

Docking compound **3** in the active site of COX-2 (Fig. 2) using the same mentioned procedure, exhibit two distinguished interactions with two different amino acids which are very similar interactions as in the case of SC-588. The first observed one is due to interaction of one of the sulfamoyl oxygen of **3** with His90 amino function. The other observed one is due to Phe518 amino function interaction with the other sulfamoyl oxygen of **3**.

Experimental

Melting points were uncorrected and were carried out by the open capillary tube method using an Electrothermal 9100 digital melting point apparatus (Stuart Scientific, Model SMM, UK). TLC was carried out using silica gel 60 F_{254} precoated sheets (E. Merck, Darmstadt, Germany) and was visualized using UV lamp (Spectroline Model CM 10, USA). IR spectra were recorded (KBr disks) on Shimadzu IR 400-91527 spectrophotometer (Shimadzu, Japan). ^{1}H-NMR spectra were recorded on a Varian EM-360L (60 MHz) and Varian GEMINI 200 (200 MHz) spectrometers (Varian, USA). Chemical shifts were expressed in δ units with tetramethylsilane as the internal standard. EI-mass spectra were recorded on JEOL, JMS-600H, mass spectrometer (JEOL, Japan), at 70 eV. Elemental analyses were performed at the Microanalytical Center, Faculty of Science, Cairo University and Assiut University.

The starting compounds **1b**, **c** (Furniss *et al*., 1989), **2a**, **b** (Aoki, 1955), **4**, **5a**, **b**, **6a**, **b** (Ahmad *et al*., 1996), **5c**

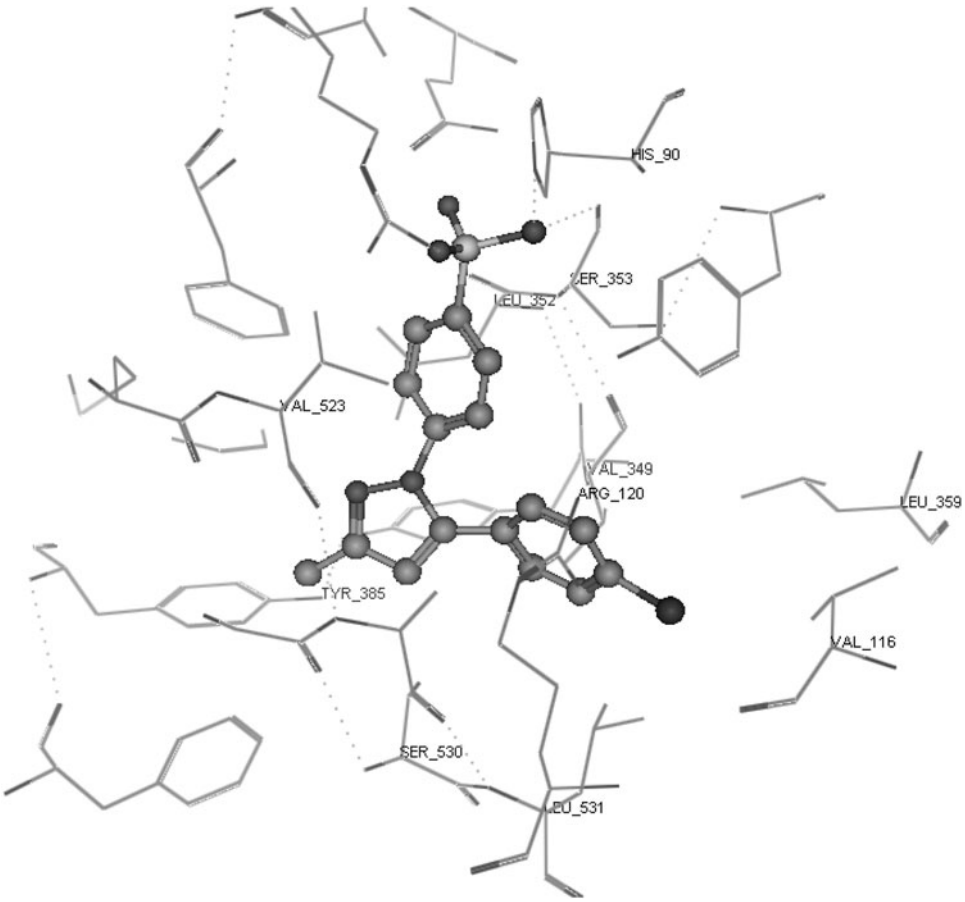


Fig. 2 Schematic representation of a docking pose of **3** in the active site of COX-2 (obtained using MOE software)

(Stanovnik and Svete, 2002) were prepared following the reported literature methods. Compound **1a** was purchased from El-Nasr Chemical Company Egypt.

General method for the preparation of 4-[5-(4-chlorophenyl)-3-methyl-1*H*-pyrazol-1-yl]-*N*-(substituted)benzenesulfonamides 3,4

A mixture of equimolar amount of the appropriate **1c**, 4-hydrazino-*N*-(substituted)benzenesulfonamide hydrochloride, sodium acetate, and glacial acetic acid (5 mmol each) in ethanol (250 ml) was stirred for 24 h at room temperature. Filtration of the mixture followed by concentrating the filtrate yielded a solid product that was collected, dried, and crystallized from ethanol (Scheme 1).

*4-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]benzenesulfonamide (**3**)*

Yield 80%, m.p. 225–226°C. IR: ν_{max}/cm^{-1} 3270, 3168 (NH_2), 1596 (C=N), 1548, 1345. ^{1}H-NMR (DMSO-d_6): δ 2.6 (s, 3H, CH_3), 7.0 (s, 1H, pyrazole *H*-4), 7.8–8.8 (m, 10H, arom. H + NH_2). MS: *m/z* (%) = 349 (M^{+2}, 31), 347 (M^+, 100), 266 (39), 232 (28), 76 (41). Anal. ($C_{16}H_{14}ClN_3O_2S$), C, H, N.

*4-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]-N-(2-thiazolyl)benzenesulfonamide (**4**)*

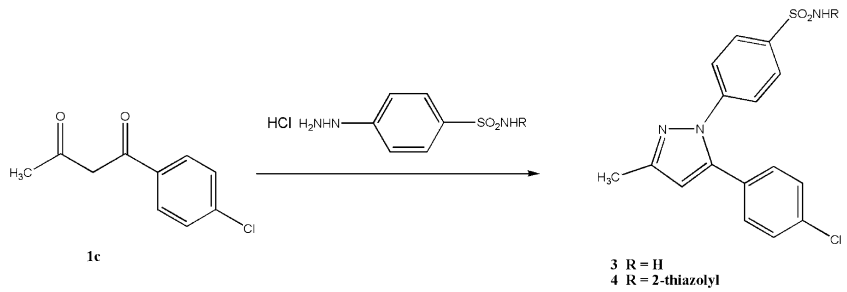
Yield 75%, m.p. 239–240°C. IR: ν_{max}/cm^{-1} 3430 (NH), 1594 (C=N), 1575, 1365. ^{1}H-NMR (DMSO-d_6): δ 2.5 (s, H, CH_3), 7.2 (s, 1H, pyrazole *H*-4), 7.4–9.2 (m, 11H, arom. H + NH). MS: *m/z* (%) = 432 (M^{+2}, 11), 430 (M^+, 29), 331 (5), 240 (6), 266 (100), 190 (15), 76 (15). Anal. ($C_{19}H_{15}ClN_4O_2S_2$), C, H, N.

*Synthesis of 2-[5-(4-chlorophenyl)-3-methyl-1H-pyrazol-1-yl] acetic acid hydrazide (**6c**)*

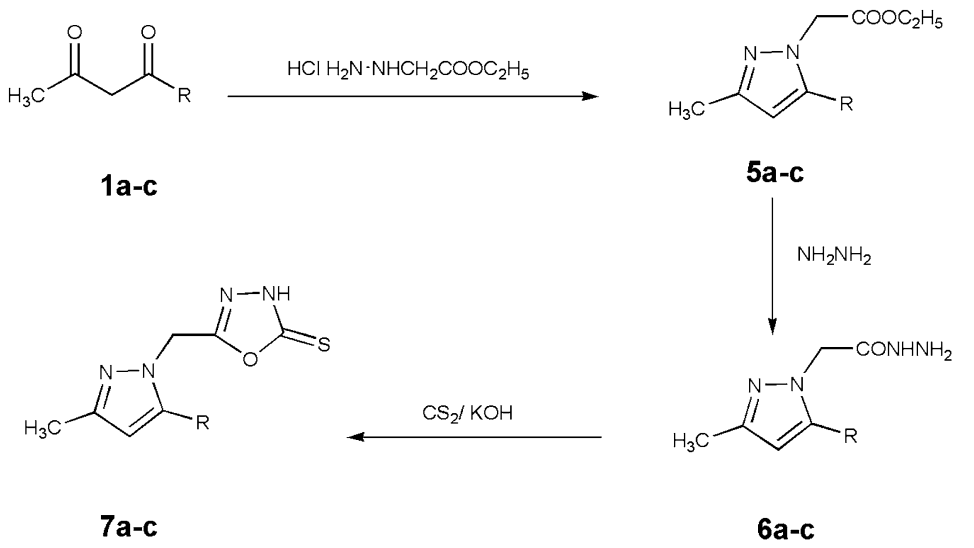
To a solution of **5c** (5 mmol) in ethanol, hydrazine hydrate 100% (5 mmol) was added dropwise with continuous stirring. The reaction mixture was then refluxed for 3 h and allowed to attain room temperature. The separated solid was filtered, dried, and crystallized from ethanol (Scheme 2).

Yield 85%, m.p. 196–197°C. IR: ν_{max}/cm^{-1} 3291, 3209 (NH), 1665 (C=O), 1629, 1539. ^{1}H-NMR (DMSO-d_6): δ 2.2

Scheme 1 Synthesis of the pyrazole compounds **3** and **4**



Scheme 2 Synthesis of the pyrazole compounds **5**, **6**, and **7**



for compouds: 1, 5, 6, 7 a; R = CH_3 , b; R = C_6H_5 , c; R = 4-ClC_6H_4

(s, 3H, CH_3), 4.3 (s, 2H, NH_2), 4.6 (s, 2H, CH_2), 6.2 (s, 1H, pyrazole *H*-4), 7.4–7.8 (m, 4H, arom. H), 9.3 (s, H, NH). MS: *m/z* (%) = 266 (M^{+2}, 17), 264 (M^+, 32), 205 (100), 192 (18), 75 (36), 68 (8). Anal. ($C_{12}H_{13}ClN_4O$), C, H, N.

General method for the preparation of 5-(3-methyl-5-substituted-1*H*-pyrazol-1-yl methyl)-3*H*-1,3,4-oxadiazole-2-thiones (**7**)

A mixture equimolar amount of the appropriate **6a–c**, potassium hydroxide (5 mmol each) and carbon disulfide (5 ml) in ethanol (25 ml) was refluxed for 12 h on a steam bath. The reaction mixture was concentrated, cooled, and neutralized with hydrochloric acid solution. The separated solid was collected, washed with water, dried, and crystallized from ethanol (Scheme 2).

*5-(3,5-Dimethyl-1H-pyrazol-1-ylmethyl)-3H-1,3,4-oxadiazole-2-thione (**7a**)*

Yield 60%, m.p. 213–215°C. IR: ν_{max}/cm^{-1} 3422 (NH), 1629, 1555, 1158. ^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 5.8 (s, 2H, CH_2), 6.5 (s, 1H, pyrazole *H*-4), 7.5 (br.s, H, NH). MS: *m/z* (%) = 210 (M^+, 57), 109 (100), 96 (9), 82 (10), 68 (23). Anal. ($C_8H_{10}N_4OS$), C, H, N.

*5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-3H-1,3,4-oxadiazole-2-thione (**7b**)*

Yield 75%, m.p. 190–192°C. IR: ν_{max}/cm^{-1} 3423 (NH), 1625, 1537, 1150. ^{1}H-NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.2 (s, 1H, pyrazole *H*-4), 7.2–7.6 (m, 6H, arom. H + NH). MS: *m/z* (%) = 272 (M^+, 100), 257 (1), 171(84), 158 (9), 144 (2), 77 (7). Anal. ($C_{13}H_{12}N_4OS$), C, H, N.

*5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl methyl]-3H-1,3,4-oxadiazole-2-thione (**7c**)*

Yield 68%, m.p. 224–225°C. IR: ν_{max}/cm^{-1} 3446 (NH), 1551, 1493 (C=N, C=C), 1151. ^{1}H-NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 5.4 (s, 2H, CH_2), 6.3 (s, 1H, pyrazole *H*-4), 7.5–7.8 (m, 4H, arom. H), 7.9 (s, 1H, NH). MS:

m/z (%) = 308 (M^{+2}, 37), 306 (M^+, 100), 205 (84), 192 (10), 178 (2), 170 (21), 128 (7). Anal. ($C_{13}H_{11}ClN_4OS$), C, H, N.

General method for the preparation of 1-[2-(3-methyl-5-substituted-1*H*-pyrazol-1-yl) acetyl]-4-substituted thiosemicarbazides (**8a**–**r**)

A mixture of equimolar amount of the appropriate **6a**–**c** and the corresponding isothiocyanate (5 mmol each) was boiled under reflux in absolute ethanol (25 ml) for 6 h. The reaction mixture was filtered while hot, concentrated and kept overnight in the refrigerator. The separated solid was collected, washed, dried, and crystallized from ethanol (Scheme 3).

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-ethyl thiosemicarbazide (**8a**)*

Yield 73%, m.p. 193–194°C. IR: ν_{max}/cm^{-1} 3423 (NH), 1741 (C=O), 1575, 1558, 1141. ^{1}H-NMR (DMSO-d_6): δ 1.3 (t, 3H, CH_2CH_3), 2.5 (s, 6H, $2CH_3$), 3.9 (q, 2H, CH_2CH_3), 5.2(s, 2H, CH_2), 6.5 (s, 1H, pyrazole *H*-4), 8.4 (s, 1H, NH), 9.7 (s, 1H, NH), 10.6 (s, 1H, NH). MS: *m/z* (%) = 255 (M^+, 18), 168 (2), 153 (3), 109 (100), 68 (6). Anal. ($C_{10}H_{17}N_5OS$), C, H, N.

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-propyl thiosemicarbazide(**8b**)*

Yield 68%, m.p. 193–195°C. IR: ν_{max}/cm^{-1} 3243, 3166 (NH), 1694 (C=O), 1620, 1542, 1154. ^{1}H-NMR (DMSO-d_6): δ 0.8 (t, 3H, $CH_2CH_2CH_3$), 1.5–1.7 (sextet, 2H, $CH_2CH_2CH_3$), 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.4 (q, 2H, CH_2 CH_2CH_3), 4.7 (s, 2H, CH_2), 5.8 (s, 1H, pyrazole *H*-4), 8.0 (s, 1H, NH), 9.2 (s, 1H, NH), 10.0 (s, 1H, NH). MS: *m/z* (%) = 269 (M^+, 26), 154 (9), 109 (100), 95 (10), 83 (3), 68 (6). Anal. ($C_{11}H_{19}N_5OS$), C, H, N.

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-cyclohexyl thiosemicarbazide (**8c**)*

Yield 70%, m.p. 189–190°C. IR: ν_{max}/cm^{-1} 3224 (NH), 1695 (C=O), 1654, 1542 (C=N, C=C), 1196.^{1}H-NMR (DMSO-d_6/D_2O): δ 1.2–1.7 (m, 11H, Cyclohexy), 2.1 (s, 3H, CH_3) 2.2(s, 3H, CH_3), 4.7 (s, 2H, CH_2), 5.8 (s, 1H, pyrazole *H*-4), 7.4(br.s, 1H, NH), 8.1(br.s, 1H, NH), 9.8(s, 1H, NH). MS: *m/z* (%) = 309 (M^+, 20), 168 (6), 153 (3), 109 (100), 95 (5), 83 (14), 68 (10), 55 (44). Anal. ($C_{14}H_{33}N_5OS$), C, H, N.

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-(3-methyl phenyl) thiosemicarbazide (**8d**)*

Yield 60%, m.p. 175–176°C. IR: ν_{max}/cm^{-1} 3341, 3297, 3240 (NH), 1704 (C=O), 1610, 1592 (C=N, C=C), 1201.^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3) 2.6 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 7.5 (s, 1H, NH), 7.8–8.3 (m, 4H, arom. H), 9.1 (br.s, 2H, 2NH). MS: *m/z* (%) = 317 (M^+, 14), 168 (25), 153 (1), 109 (100), 95 (7), 68 (10), 55 (5). Anal. ($C_{15}H_{19}N_5OS$), C, H, N.

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-(4-methoxyphenyl) thiosemicarbazide (**8e**)*

Yield 76%, m.p. 188–190°C. IR: ν_{max}/cm^{-1} 3538, 3241 (NH), 1694 (C=O), 1609, 1541, 1202 ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.6 (s, 3H, CH_3) 4.1 (s, 3H, OCH_3), 5.2 (s, 2H, CH_2), 6.3 (s, 1H, pyrazole *H*-4) 7.3–8.1 (m, 4H, arom. H), 10.5 (s, 2H, 2NH), 11.1 (s, 1H, NH). Anal. ($C_{15}H_{19}N_5O_2S$), C, H, N.

*1-[2-(3,5-Dimethyl-1H-pyrazol-1-yl) acetyl]-4-(4-nitrophenyl) thiosemicarbazide (**8f**)*

Yield 86%, m.p. 222–223°C. IR: ν_{max}/cm^{-1} 3270 (NH), 1702 (C=O), 1650, 1619,1204. ^{1}H-NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 7.5–8.4 (m, 4H, arom. H), 10.5 (s, 2H, 2NH), 11.2 (s, 1H, NH). Anal. ($C_{14}H_{16}N_6O_3S$), C, H, N.

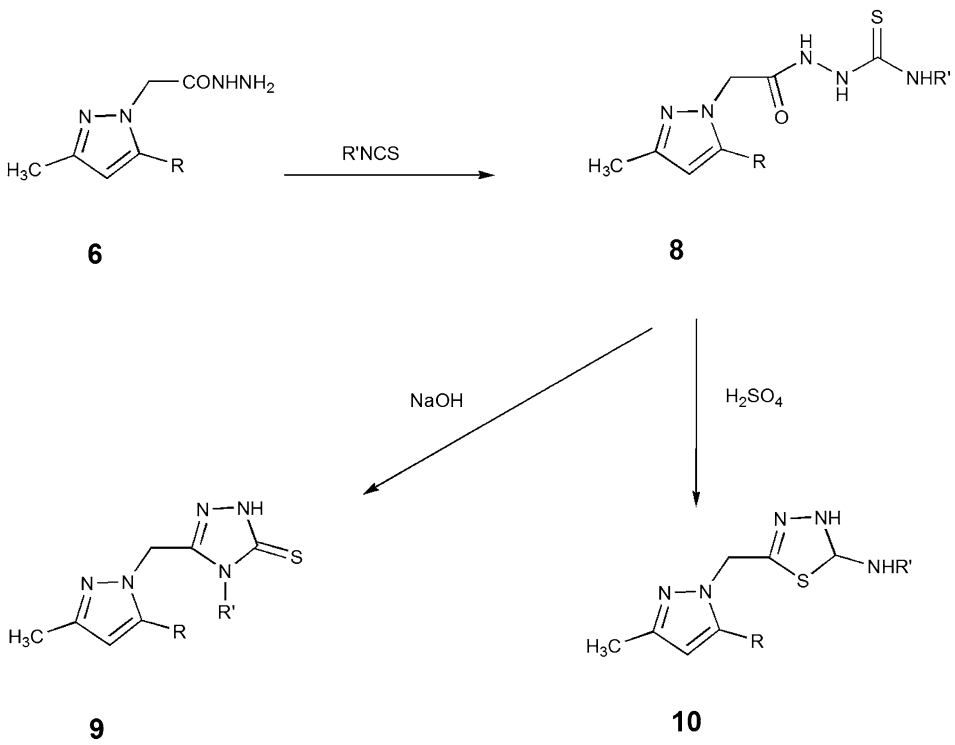
*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl) acetyl]-4-ethyl thiosemicarbazide (**8g**)*

Yield 60%, m.p. 171–172°C. IR: ν_{max}/cm^{-1} 3264 (NH), 1688 (C=O), 1548, 1499 (C=N, C=C), 1198.^{1}H-NMR (DMSO-d_6): δ 1.3 (t, 3H, CH_2CH_3), 2.6 (s, 3H, CH_3) 3.9 (q, 3H, CH_2CH_3), 5.3 (s, 2H, CH_2), 6.8 (s, 1H, pyrazole *H*-4), 8.1–8.8 (m, 5H, arom. H), 10.0 (s, H, NH), 10.3 (br.s, 1H, NH), 11.1(s, 1H, NH). MS: *m/z* (%) = 317 317 (M^+, 20), 230 (10), 171 (100), 157 (5), 144 (4), 77 (11), 68 (2). Anal. ($C_{15}H_{19}N_5OS$), C, H, N.

*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl) acetyl]-4-propyl thiosemicarbazide (**8h**)*

Yield 60%, m.p. 171–172°C. IR: ν_{max}/cm^{-1} 3268 (NH), 1687 (C=O), 1550, 1497, 1195 .^{1}H-NMR (DMSO-d_6): δ 0.9 (t, 3H, $CH_2CH_2CH_3$), 1.7 (m, 2H, $CH_2CH_2CH_3$) 2.4 (s,

Scheme 3 Synthesis of the pyrazole compounds **8**, **9**, and **10**



Compd. No.	R	R'	Compd. No.	R	R'
8a **9a**	CH_3	C_2H_5	**8j** **9j**	C_6H_5	3-H_3C-C_6H_4
8b **9b**	CH_3	C_3H_7	**8k** **9k**	C_6H_5	4-H_3CO-C_6H_4
8c **9c**	CH_3	C_6H_{11}	**8l**	C_6H_5	4-O_2N-C_6H_4
8d **9d**	CH_3	3-H_3C-C_6H_4	**8m** **9m**	4-Cl-C_6H_4	C_2H_5
8e	CH_3	4-H_3CO-C_6H_4	**8n**	4-Cl-C_6H_4	C_6H_{11}
8f **9f** **10f**	CH_3	4-O_2N-C_6H_4	**8o** **9o**	4-Cl-C_6H_4	C_6H_5
8g **9g**	C_6H_5	C_2H_5	**8p** **9p**	4-Cl-C_6H_4	3-H_3C-C_6H_4
8h **9h** **10h**	C_6H_5	C_3H_7	**8q** **9q**	4-Cl-C_6H_4	4-H_3CO-C_6H_4
8i	C_6H_5	C_6H_{11}	**8r** **10r**	4-Cl-C_6H_4	4-O_2N-C_6H_4

3H, CH_3) 3.7 (q, 2H, $CH_2CH_2CH_3$), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 8.4(m, 5H, arom-H), 9.9 (br.s, 2H, 2NH), 10.8 (s, 1H, NH). MS: *m/z* (%) = 317 (M^+, 20), 230 (10), 171 (100), 157 (5), 144 (4), 77 (11), 68 (2). Anal. ($C_{16}H_{21}N_5OS$), C, H, N.

*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl)acetyl]-4-cyclohexyl thiosemicarbazide (**8i**)*

Yield 78%, m.p. 192–194°C. IR: ν_{max}/cm^{-1} 3281 (NH), 1697 (C=O), 1551, 1496, 1224.^{1}H-NMR (DMSO-d_6): δ

1.2–1.8 (m, 11H, cyclohexyl), 2.2 (s, 3H, CH_3), 4.7 (s, 2H, CH_2), 6.2 (s, 1H, pyrazole *H*-4), 7.4 (m, 5H, arom. H), 9.3 (s, 1H, NH), 9.4 (s, 1H, NH), 10.1 (s, 1H, NH). Anal. ($C_{19}H_{25}N_5OS$), C, H, N.

*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl) acetyl]-4-(3-methylphenyl) thiosemicarbazide (**8j**)*

Yield 60%, m.p. 157–158°C. IR: ν_{max}/cm^{-1} 3245 (NH), 1684 (C=O), 1593, 1549, 1212 .^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 7.5–8.4 (m, 9H, arom. H), 9.4 (br.s, 1H, NH), 9.6 (s, 1H, NH), 10.4 (s, 1H, NH). Anal. ($C_{20}H_{21}N_5OS$), C, H, N.

*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl)acetyl]-4-(4-methoxyphenyl) thiosemicarbazide (**8k**)*

Yield 75%, m.p. 174–176°C. IR: ν_{max}/cm^{-1} 3295 (NH), 1674 (C=O), 1617, 1551, 1173. ^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 4.2 (s, 3H, OCH_3), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 7.3–8.4 (m, 9H, arom. H), 10.3 (br.s, 2H, 2NH), 11.3 (s, 1H, NH). Anal. ($C_{20}H_{21}N_5O_2S$), C, H, N.

*1-[2-(3-Methyl-5-phenyl-1H-pyrazol-1-yl)acetyl]-4-(4-nitrophenyl) thiosemicarbazide (**8l**)*

Yield 85%, m.p. 180–181°C. IR: ν_{max}/cm^{-1} 3218 (NH), 1693 (C=O), 1597, 1555, 1188 .^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 5.3 (s, 2H, CH_2), 6.8 (s, 1H, pyrazole *H*-4), 8.1–9.2 (m, 9H, arom. H), 10.8 (br.s, 2H, 2NH), 11.2 (s, 1H, NH). Anal. ($C_{19}H_{18}N_6O_3S$), C, H, N.

*1-{2-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]acetyl}-4-ethylthiosemicarbazide (**8m**)*

Yield 70%, m.p. 204–205°C. IR: ν_{max}/cm^{-1} 3256, 3158 (NH), 1696 (C=O), 1554, 1493, 1229.^{1}H-NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_2CH_3), 2.5 (s, 3H, CH_3), 3.8 (q, 2H, CH_2CH_3), 5.2 (s, 2H, CH_2), 6.6 (s, 1H, pyrazole *H*-4), 7.4–7.7 (m, 4H, arom. H), 8.2 (s, 1H, NH), 9.3 (s, 1H, NH), 10.0 (s, 1H, NH) Anal. ($C_{15}H_{18}ClN_5OS$), C, H, N.

*1-{2-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]acetyl}-4-cyclohexyl thiosemicarbazide (**8n**)*

Yield 60%, m.p. 114–115°C. IR: ν_{max}/cm^{-1} 3273 (NH), 1703 (C=O), 1620, 1551, 1224. ^{1}H-NMR (DMSO-d_6): δ 0.9–2.2 (m, 11H, cyclohexyl), 2.4 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.8 (s, 1H, pyrazole *H*-4), 8.2–8.9 (m, 4H, arom. H), 10.2 (br.s, 2H, 2NH), 11.2 (s, 1H, NH)**.** MS *m/z* (%) = 407 (M^{+2}, 4), 405 (M^+, 14), 306 (6), 264 (53), 205 (100), 193 (13), 68 (5). Anal. ($C_{19}H_{24}ClN_5OS$), C, H, N.

*1-{2-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]acetyl}-4-phenylthiosemicarbazide (**8o**)*

Yield 76%, m.p. 186–187°C. IR: ν_{max}/cm^{-1} 3359 (NH), 1701 (C=O), 1598, 1540, 1190. ^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 7.8–8.6 (m, 9H, arom. H), 10.4 (s, 2H, 2NH), 11.1 (s, 1H, NH). Anal. ($C_{19}H_{18}ClN_5OS$), C, H, N.

*1-{2-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]acetyl}-4-(3-methylphenyl) thiosemicarbazide (**8p**)*

Yield 92%, m.p. 160–162°C. IR: ν_{max}/cm^{-1} 3209 (NH), 1690 (C=O), 1607, 1539, 1201. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.3 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 7.5–8.5 (m, 8H, arom. H), 10.3 (s, 2H, 2NH), 10.9 (br.s, 1H, NH). Anal. ($C_{20}H_{20}ClN_5OS$), C, H, N.

*1-{2-[-5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-yl]acetyl}-4-(4-methoxyphenyl) thiosemicarbazide (**8q**)*

Yield 75%, m.p. 167–169°C. IR: ν_{max}/cm^{-1} 3219 (NH), 1693 (C=O), 1605, 1512, 1177. ^{1}H-NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 4.2 (s, 3H, OCH_3), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 7.4–8.6 (m, 9H, arom. H + NH), 10.4 (s, 2H, 2NH). Anal. ($C_{20}H_{20}ClN_5O_2S$), C, H, N.

*1-{2-[-5-(4-Chlorophenyl)3-methyl-1H-pyrazol-1-yl]acetyl}-4-(4-nitrophenyl) thiosemicarbazide (**8r**)*

Yield 80%, m.p. 180–181°C. IR: ν_{max}/cm^{-1} 3191 (NH), 1690 (C=O), 1599, 1549, 1201. ^{1}H-NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.7 (s, 1H, pyrazole *H*-4), 8.0–9.1 (m, 8H, arom. H), 10.7 (s, 3H, 3NH). Anal. ($C_{19}H_{17}ClN_6O_3S$), C, H, N.

General method for the preparation of 5-(3-methyl-5-substituted-1*H*-pyrazol-1-ylmethyl)-4-substituted-2*H*-1,2,4-triazole-3-thiones (**9a–d**, **f–h**, **j**, **k**, **m**, **o–q**)

A 2N sodium hydroxide solution (2 ml) was added to a solution of the appropriate **8a–d**, **f–h**, **j**, **k**, **m**, and **o–q** (5 mmol) in ethanol (50 ml). The reaction mixture was then refluxed for 7 h, cooled, filtered, and the filtrate was acidified with 2N hydrochloric acid. The separated solid was collected, washed, and crystallized from ethanol (Scheme 3).

*5-(3,5-Dimethyl-1H-pyrazol-1-yl methyl)-4-ethyl-2H-1,2,4-triazole-3-thione (**9a**)*

Yield 65%, m.p. 201–203°C. IR: ν_{max}/cm^{-1}3442 (NH), 1558, 1510, 1186. ^{1}H-NMR (DMSO-d_6): δ1.2 (t, 3H, CH_2CH_3), 2.5 (s, 3H, CH_3), 4.4 (q, 2H, CH_2CH_3), 5.8 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 14.4 (s, 1H, NH). MS: *m/z* (%) = 237 (M^+, 100), 209 (19), 109 (68), 95 (46), 68 (24). Anal. ($C_{10}H_{15}N_5S$), C, H, N.

*5-(3,5-Dimethyl-1H-pyrazol-1-yl methyl)-4-propyl-2H-1,2,4-triazole-3-thione (**9b**)*

Yield 73%, m.p. 184–186°C. IR: ν_{max}/cm^{-1} 3368 (NH), 1644, 1564, 1141. ^{1}H-NMR (DMSO-d_6): δ0.8 (t, 3H, $CH_2CH_2CH_3$), 1.4 (m, 2H, $CH_2CH_2CH_3$), 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.8 (t, 2H, $CH_2CH_2CH_3$), 5.4 (s, 2H, CH_2), 5.9 (s, 1H, pyrazole *H*-4), 13.8 (s, 1H, NH). MS: *m/z* (%) = 251 (M^+, 85), 208 (100), 109 (59), 95 (46), 68 (9). Anal. ($C_{11}H_{17}N_5S$), C, H, N.

*5-(3,5-Dimethyl-1H-pyrazol-1-yl methyl)-4-cyclohexyl-2H-1,2,4-triazole-3-thione (**9c**)*

Yield 82%, m.p. 268–269°C. IR: ν_{max}/cm^{-1} 3422 (NH), 1650, 1564, 1184. ^{1}H-NMR (DMSO-d_6): δ 0.9–2.1 (m, 11H, cyclohexyl), 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 5.9 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 13.5 (s, 1H, NH). MS: *m/z* (%) = 291 (M^+, 40), 208 (100), 109 (35), 95 (49), 68 (6), 55 (45). Anal. ($C_{14}H_{21}N_5S$), C, H, N.

*5-(3,5-Dimethyl-1H-pyrazol-1-ylmethyl)-4-(3-methylphenyl)-2H-1,2,4-triazole-3-thione (**9d**)*

Yield 78%, m.p. 231–232°C. IR: ν_{max}/cm^{-1} 3446 (NH), 1578, 1519, 1228. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 7.5–8.3 (m, 4H, arom. H), 9.1 (br.s, 1H, NH). MS: *m/z* (%) = 299 (M^+, 100), 208 (1), 109 (49), 95 (28), 68 (10). Anal. ($C_{15}H_{17}N_5S$), C, H, N.

*5-(3,5-Dimethyl-1H-pyrazol-1-yl methyl)-4-(4-nitrophenyl)-2H-1,2,4-triazole-3-thione (**9f**)*

Yield 50%, m.p. 226–227°C. IR: ν_{max}/cm^{-1} 3242 (NH), 1600, 1233. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 8.2–9.2 (m, 4H, arom. H), 11.0 (s, 1H, NH). Anal. ($C_{14}H_{14}N_6O_2S$), C, H, N.

*5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-4-ethyl-2H-1,2,4-triazole-3-thione (**9g**)*

Yield 80%, m.p. 174–175°C. IR: ν_{max}/cm^{-1} 3435 (NH), 1575, 1551, 1187. ^{1}H-NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_2CH_3), 2.5 (s, 3H, CH_3), 4.3 (q, 2H, CH_2CH_3), 5.8 (s, 2H, CH_2), 6.6 (s, 1H, pyrazole *H*-4), 8.1 (m, 5H, arom. H), 12.9 (s, 1H, NH). Anal. ($C_{15}H_{17}N_5S$), C, H, N.

*5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-4-propyl-2H-1,2,4-triazole-3-thione (**9h**)*

Yield 78%, m.p. 108–110°C. IR: ν_{max}/cm^{-1} 3422 (NH), 1653, 1576, 1186. ^{1}H-NMR (DMSO-d_6): δ 0.9(t, 3H, $CH_2CH_2CH_3$), 1.7(m, 2H, $CH_2CH_2CH_3$) 2.5 (s, 3H, CH_3) 4.3 (t, 2H, $CH_2CH_2CH_3$), 5.2 (s, 2H, CH_2), 5.9 (s, 2H, CH_2), 6.8 (s, 1H, pyrazole *H*-4), 8.2(m, 5H, arom-H), 12.9(s, 1H, NH). Anal. ($C_{16}H_{19}N_5S$), C, H, N.

*5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-4-(3-methylphenyl)-2H-1,2,4-triazole-3-thione (**9j**)*

Yield 82%, m.p. 188–189°C. IR: ν_{max}/cm^{-1} 3447 (NH), 1607, 1592, 1177. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.5 (s, 2H, CH_2), 6.4 (s, 1H, pyrazole *H*-4), 7.2–8.1 (m, 9H, arom. H), 13.8 (s, 1H, NH). Anal. ($C_{20}H_{19}N_5S$), C, H, N.

*5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-4-(4-methoxyphenyl)-2H-1,2,4-triazole-3-thione (**9k**)*

Yield 75%, m.p. 190–192°C. IR: ν_{max}/cm^{-1} 3422 (NH), 1610, 1582, 1174. ^{1}H-NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.2 (s, 2H, CH_2), 6.0 (s, 1H, pyrazole *H*-4), 6.8–7.4 (m, 9H, arom. H), 13.8 (s, 1H, NH). Anal. ($C_{20}H_{19}N_5OS$), C, H, N.

*5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-ylmethyl]-4-ethyl-2H-1,2,4-triazole-3-thione (**9m**)*

Yield 63%, m.p. 189–190°C. IR: ν_{max}/cm^{-1} 3438 (NH), 1569, 1499, 1193. ^{1}H-NMR (DMSO-d_6): δ 0.9 (t, 3H, CH_2CH_3), 2.1 (s, 3H, CH_3), 3.9 (q, 2H, CH_2CH_3), 5.4 (s, 2H, CH_2), 6.3 (s, 1H, pyrazole *H*-4), 7.5–7.8 (m, 4H, arom. H), 13.7 (s, 1H, NH). MS: *m/z* (%) = 335 (M^{+2}, 37), 333 (M^+, 100), 304 (17), 205 (25), 192 (20), 101 (3). Anal. ($C_{15}H_{16}ClN_5S$), C, H, N.

*5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-ylmethyl]-4-phenyl-2H-1,2,4-triazole-3-thione (**9o**)*

Yield 63%, m.p. 227–229°C. IR: ν_{max}/cm^{-1} 3442 (NH), 1567, 1488, 1193. ^{1}H-NMR (DMSO-d_6): δ 2.1 (s, 3H,

CH_3), 5.2 (s, 2H, CH_2), 6.0 (s, 1H, pyrazole *H*-4), 7.0–7.5 (m, 9H, arom. H), 13.9 (s, 1H, NH). MS: *m/z* (%) = 383 (M^{+2}, 10), 381 (M^+, 36), 204 (16), 192 (21), 177 (2), 77 (7). Anal. ($C_{19}H_{16}ClN_5S$), C, H, N.

*5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-ylmethyl]-4-(3-methylphenyl)-2H-1,2,4-triazole-3-thione (**9p**)*

Yield 78%, m.p. 218–219°C. IR: ν_{max}/cm^{-1} 3445 (NH), 1604, 1580, 1177. ^{1}H-NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 2.2 (s, 3H, CH_3), 5.2 (s, 2H, CH_2), 6.0 (s, 1H, pyrazole *H*-4), 6.7–7.4 (m, 8H, arom. H), 15.2 (br.s, 1H, NH). MS: *m/z* (%) = 397 (M^{+2}, 26), 395 (M^+, 100), 335 (11), 203 (22), 192 (32), 91 (22). Anal. ($C_{20}H_{18}ClN_5S$), C, H, N.

*5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-ylmethyl]-4-(4-methoxyphenyl)-2H-1,2,4-triazole-3-thione (**9q**)*

Yield 78%, m.p. 166–167°C. IR: ν_{max}/cm^{-1} 3422 (NH), 1607, 1514, 1174. ^{1}H-NMR (DMSO-d_6): δ 2.6 (s, 3H, CH_3), 4.2 (s, 3H, OCH_3), 5.6 (s, 2H, CH_2), 6.6 (s, 1H, pyrazole *H*-4), 7.2–8.4 (m, 9H, arom. H + NH). MS: *m/z* (%) = 413 (M^{+2}, 43), 411 (M^+, 100), 381 (2), 205 (18), 192 (29). Anal. ($C_{20}H_{18}ClN_5OS$), C, H, N.

General method for the preparation of [5-(3-methyl-5-substituted-1*H*-pyrazol-1-ylmethyl)-1,3,4-thiadiazol-2-yl]-substituted amines (**10f**, **h**, **r**)

Compounds **8f**, **h**, **r** (5 mmol) were separately added portion wise with constant stirring to a concentrated sulfuric acid solution (40 ml) cooled at 0°C. After complete addition, the reaction mixture was stirred for an additional 3 h at room temperature then left overnight at the same temperature. The solution was then poured onto ice-cooled water and the separated solid was filtered, washed, dried, and crystallized from acetic acid–water mixture as 1:2 v/v (Scheme 3).

*[5-(3,5-Dimethyl-1H-pyrazol-1-ylmethyl)-1,3,4-thiadiazol-2-yl]-(4-nitrophenyl)amine (**10f**)*

Yield 54%, m.p. 226–227°C. IR: ν_{max}/cm^{-1} 3436 (NH), 1602, 1581. ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.8 (s, 2H, CH_2), 6.2 (s, 1H, pyrazole *H*-4), 8.1–9.0 (m, 4H, arom. H), 11.8 (br.s, 1H, NH). MS: *m/z* (%) = 330 (M^+, 100), 236 (31), 181 (13), 149 (10), 109 (38), 95 (36). Anal. ($C_{14}H_{14}N_6O_2S$), C, H, N.

*[5-(3-Methyl-5-phenyl-1H-pyrazol-1-ylmethyl)-1,3,4-thiadiazol-2-yl]-propylamine (**10h**)*

Yield 54%, m.p. 226–227°C. IR: ν_{max}/cm^{-1} 3441 (NH), 1579, 1523. ^{1}H-NMR (DMSO-d_6): δ 0.9 (t, 3H, $CH_2CH_2CH_3$), 1.6 (m, 2H, $CH_2CH_2CH_3$), 2.2 (s, 3H, CH_3), 3.2 (m, 2H, $CH_2CH_2CH_3$), 5.4 (s, 2H, CH_2), 6.2 (s, 1H, pyrazole *H*-4), 7.4–7.6 (m, 5H, arom. H), 7.8 (s, 1H, NH). Anal. ($C_{16}H_{19}N_5S$), C, H, N.

*{5-[5-(4-Chlorophenyl)-3-methyl-1H-pyrazol-1-ylmethyl]-1,3,4-thiadiazol-2-yl}-(4-nitrophenyl)amine (**10r**)*

Yield 54%, m.p. 248–250°C. IR: ν_{max}/cm^{-1} 3405 (NH), 1628, 1593). ^{1}H-NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 5.6 (s, 2H, CH_2), 6.3 (s, 1H, pyrazole *H*-4), 7.6–8.3 (m, 8H, arom. H), 11.1 (br. s, 1H, NH). MS: *m/z* (%) = 428 (M^{+2}, 7), 426 (M^+, 19), 235 (13), 192 (100), 109 (38), 95 (36), 68 (5). Anal. ($C_{19}H_{15}ClN_6O_2S$), C, H, N.

Anti-inflammatory activity

Compounds **3**, **4**, **7a–c**, **8d–f**, **9d**, **9f** and **10f**, **h**, **r** were screened for their in vivo anti-inflammatory activity by the carrageenan-induced paw edema standard method. Adult albino rats of either sex (pregnant female animals were excluded) weighing 160–190 g were divided into 16 group of 6 animals each. To reduce the variability of edema response, rats were fasted overnight, then on the next day (day of experiment), animals were uniformly hydrated by giving 3 ml of water per rat orally. Indomethacin and celecoxib (reference standards) and the tested compounds (100 mg/kg body weight) were suspended in saline solution by the aid of few drops of Tween 80 (to improve wettability of particles) and given orally 1 h before induction of inflammation. The control group was given saline solution containing few drops of Tween 80.

Carrageenan paw edema was induced according to a modified method (Winter *et al*.) by subcutaneous injection of 1% solution of carrageenan in saline (0.1 ml/rat) into the subplanter region of the right hind paw of rats. The thickness of rat paw was measured by mercury digital micrometer at different time intervals, after 0, 1, 2, and 3 h of carrageenan injection. The edema was determined from the difference between the thickness of injected and non-injected paws. Data were collected, checked, revised, and analyzed. Quantitative variables from normal distribution were expressed as mean ± standard error (SE). The significant difference between groups was tested by using one-way ANOVA followed by Dunnett's *t* test at $P < 0.05$ and <0.01.

The anti-inflammatory activity was expressed as percentage inhibition of edema thickness in treated animals in comparison with the control group (Table 1).

$$\% \text{ Inhibition of edema} = \frac{(V_R - V_L)\text{control} - (V_R - V_L)\text{treated}}{(V_R - V_L)\text{control}} \times 100$$

where V_R is the average right paw thickness; V_L is the average left paw thickness.

Ulcerogenic liability

The ulcerogenic liability was determined in albino rats following the reported method (Barsoum *et al*., 2006, 2009). Rats of either sex (pregnant female rats were excluded) weighing 120–140 g were divided into five groups of five animals each. The animals were fasted 18 h before drug administration. Indomethacin (reference standard) and the tested compounds **3**, **8f**, **10f** (100 mg/kg body weight) were suspended in saline by the aid of few drops of Tween 80 and were administered once orally for three successive days to fasted rats. The control group animals were given only saline with few drops of Tween 80. One hour following the dose, the animals were sacrificed and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10×) for the presence of lesions and erosions. The ulcer index was calculated (Table 2) and the degree of ulcerogenic effect was expressed in terms of:

1. Percentage incidence of ulcer divided by 10.
2. Average number of ulcers per stomach.
3. Average severity of ulcers.

The ulcer index is the value that resulted from the sum of the above three values.

Docking studies

Computer-assisted simulated docking experiments were carried out under an MMFF94X forcefield using Molecular Operating Environment (MOE Dock 2008) software, Chemical Computing Group, Montréal, Canada.

Docking protocol

The coordinates from the X-ray crystal structure of human COX-2 used in this simulation were obtained from the Protein Data Bank (PDB ID: 1CX2), where the active site is bound to the selective COX-2 inhibitor SC-588. The ligand molecules were constructed using the builder module and were energy minimized. The active site of COX-2 was generated using the MOE-Alpha Site Finder, and then ligands were docked within this active site using the MOE-Dock. The lowest energy conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with COX-2 were determined.

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Conflict of interest None.

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