Med Chem Res
DOI 10.1007/s00044-011-9853-4

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory–antibacterial agents

Pawan Kumar · Navneet Chandak · Pawan Kaushik · Chetan Sharma · Dhirender Kaushik · Kamal R. Aneja · Pawan K. Sharma

Received: 9 March 2011 / Accepted: 28 October 2011

Abstract The present article describes the synthesis of two novel series of thiosemicarbazones **3** and thiazolylhydrazinomethylidenepyrazoles **5**. All the newly synthesized target compounds (**3a–e** and **5a–o**) were screened for their in vivo anti-inflammatory (AI) activity using carrageenan-induced rat paw edema assay and in vitro antibacterial activity against two Gram-positive and two Gram-negative bacteria. Eight compounds (**3b–d**, **5b**, **5e**, **5f**, **5i**, and **5o**) showed consistently excellent AI activity (≥70% inhibition), at 3 and 4 h after the carrageenan injection, comparable to that of standard drug indomethacin (78%) whereas the remaining twelve compounds have shown significant activity with 57–75% inhibition after 3 h and 56–63% inhibition after 4 h. All the tested compounds showed moderate antibacterial properties.

Keywords Thiosemicarbazones · Thiazoles · Pyrazoles · Anti-inflammatory activity · Antibacterial activity

Introduction

The development of an effective therapeutic agent for the management of inflammation has undergone continual evolution leading to the emergence of more efficacious classes of drugs. Since the discovery of aspirin, much efforts have been devoted to the development of non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most widely prescribed medication in clinical practice despite their well documented renal and gastrointestinal (GI) side effects. Conventional NSAIDs exert non-selective inhibition (Dannhardt and Keifer, 2001) of COX enzymes, the agents which catalyze the rate-limiting step in the formation of prostanoids from arachidonic acid. Such indiscriminate inhibition of COX-1 as well as COX-2 has been blamed for high incidence of GI irritation or, in the worst case, development of life threatening GI ulcers and bleeding in long term users of NSAIDs. Consequently, a second generation of NSAIDs has been developed which selectively inhibit COX-2. Being selective COX-2 inhibitors, these are expected to achieve the same anti-inflammatory efficacy as traditional NSAIDs but minimize the risk of unwanted GI complications. Though the selective COX-2 inhibitors have minimal toxicity in the gastrointestinal tract, these agents can produce severe side effects in renal, hepatic, and cardiovascular systems. The recent withdrawal of valdecoxib (Nussmeier *et al.*, 2005), and rofecoxib (Scheen, 2004) has focused attention on the adverse cardiovascular effects of selective COX-2 inhibitors. Thus, search for novel anti-inflammatory drugs with minimal GI side effects and high safety margin is still warranted.

Pyrazole moiety makes the core structure of various drugs such as difenamizole (Kameyama *et al.*, 1978; Kameyama and Nabeshima, 1978), celecoxib (Penning *et al.*, 1997) tepoxalin, (Anderson *et al.*, 1990) etc. Besides this, there are several reports in the literature on the anti-inflammatory (Bekhit *et al.*, 2003; Banoglu *et al.*, 2004; Bekhit *et al.*, 2005; Rosati *et al.*, 2007; Szabó *et al.*, 2008) and antimicrobial properties (Foks *et al.*, 2005; Shamroukh *et al.*, 2007;

P. Kumar · N. Chandak · P. K. Sharma (✉)
Department of Chemistry, Kurukshetra University, Kurukshetra 136119, India
e-mail: pksharma@kuk.ac.in

P. Kaushik · D. Kaushik
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra 136119, India

C. Sharma · K. R. Aneja
Department of Microbiology, Kurukshetra University, Kurukshetra 136119, India

Published online: 19 November 2011

Prakash *et al.*, 2008) of pyrazoles. However studies investigating the potential of pyrazole derivatives as dual antimicrobial–anti-inflammatory agents has only recently been initiated (Bekhit and Fahmy, 2003; Bekhit and Abdel-Azeim, 2004; Bekhit *et al.*, 2005; Bekhit *et al.*, 2006; Bekhit *et al.*, 2009). Thiazoles and their derivatives are also known to exhibit antimicrobial (Bondock *et al.*, 2007; Karegoudar *et al.*, 2008) as well as anti-inflammatory activity (Kalkhambkar *et al.*, 2007; Kouatly *et al.*, 2009; Giri *et al.*, 2009). Since the combination of pharmacophores on the same scaffold is a well established approach to the synthesis of more potent drugs (Pillai *et al.*, 2003; Venkatachalam *et al.*, 2006), we decided to incorporate pyrazole moiety and thiazole ring in the same molecule while retaining the benzenesulfonamide group in an effort to synthesize new compounds with dual anti-inflammatory–antibacterial potential.

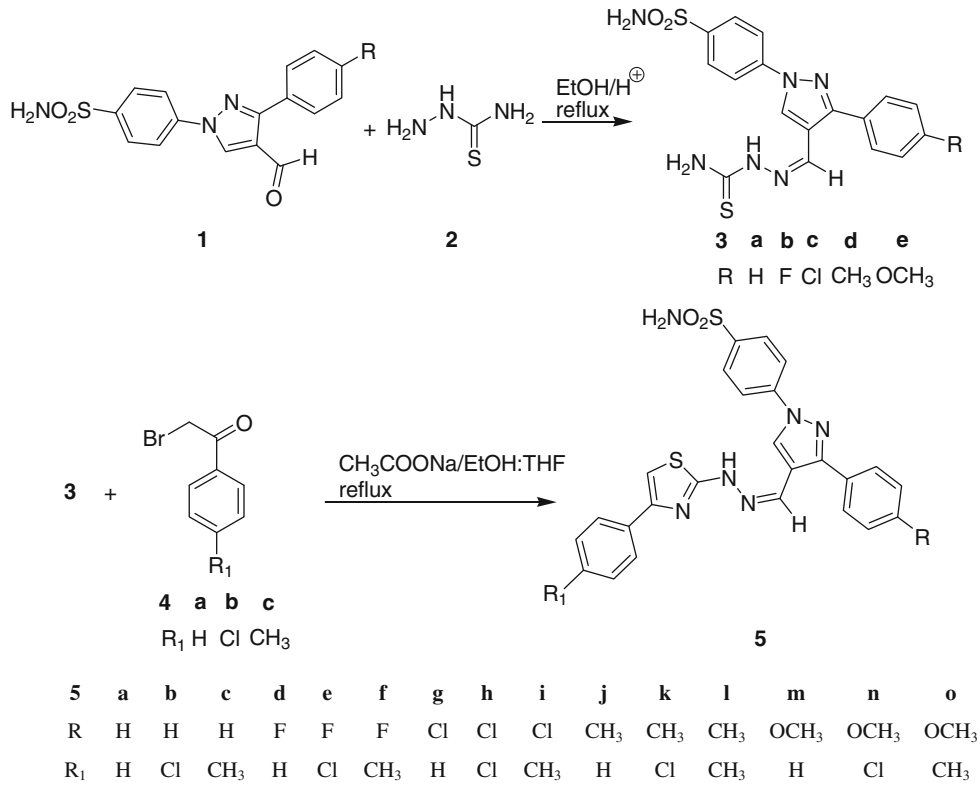
Motivated by these findings coupled with our ongoing program in the field of pyrazoles and other heterocyclic compounds (Sawhney and Sharma, 1993; Sharma and Sawhney, 1993; Sharma and Sawhney, 1997; Sharma *et al.*, 1998, 2010, 2011), as anti-inflammatory agents, it was decided to synthesize two novel series of thiosemicarbazones **3** and thiazolylhydrazinomethylidenepyrazoles **5** with a potential to act as dual anti-inflammatory–antibacterial agents with minimal GI side effects and high safety margin.

Results and discussion

Chemistry

The reaction of α-haloketones with a thioamide has been the most important method for the thiazole synthesis ever since it was introduced by Hantzsch and Weber (1887). Corresponding to the Hantzsch thiazole synthesis, the present synthesis of thiazolylhydrazinomethylidenepyrazoles **5** consists of the condensation of α-bromoketones **4** with thiosemicarbazones **3** in refluxing EtOH:THF in the presence of sodium acetate (Scheme 1).

Accordingly 4-formylpyrazoles (**1a–e**) (Bekhit *et al.*, 2009; Sharma *et al.*, 2011) were treated with thiosemicarbazide **2** in the presence of catalytic amount of acetic acid to afford the corresponding thiosemicarbazones **3** which on subsequent reaction with various α-bromoketones **4** afforded the target thiazolylhydrazinomethylidenepyrazoles **5**. Spectral data (^{1}H NMR, ^{13}C NMR, IR and mass) of the newly synthesized compounds were in full agreement with the proposed structures. In general, the characteristic signals in ^{1}H NMR of target thiosemicarbazones **3** are the presence of three exchangeable singlets in the range of δ 11.41–11.43, δ 8.26–8.32 and δ 7.77–7.82 corresponding to SH, NH and =NH protons indicating that in solution $CSNH_2$ moiety may exist in its tautomeric form



Scheme 1 Synthesis of target thiosemicarbazones **3** and thiazolylhydrazinomethylidenepyrazoles **5**

(HS–C=NH). Another exchangeable singlet integrating for two protons in the range of δ 7.46–7.49 was ascribed to NH_2 of SO_2NH_2 group. The ^1H NMR spectra of thiazolylhydrazinomethylidenepyrazoles **5** displayed an exchangeable singlet in the range of δ 11.99–12.05 corresponding to NH besides a singlet for NH_2 of SO_2NH_2 group in the range of δ 7.44–7.49. A singlet in the range of δ 7.20–7.40 is attributed to C5-proton of thiazole ring. In some of the compounds, this C5-proton of thiazole is merged with other aromatic protons.

Biological evaluation

In vivo anti-inflammatory activity

All the newly synthesized thiosemicarbazones **3a–e** and thiazolylhydrazinomethylidenepyrazoles **5a–o** were evaluated for their in vivo anti-inflammatory activity by carrageenan induced rat paw edema method (Winter *et al.*, 1962). The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). Each test compound was dosed orally (50 mg/kg body weight) 30 min prior to induction of inflammation by carrageenan injection. Indomethacin was used as a reference anti-inflammatory drug at a dose of 10 mg/kg, i.p. The anti-inflammatory activity was then calculated at hourly intervals 1–4 h after induction and presented in Table 1 as the mean paw volume (ml) as well as the percentage anti-inflammatory activity (AI%).

Among twenty compounds (**3a–e** and **5a–o**) tested, eight compounds (**3b–d**, **5b**, **5e**, **5f**, **5i**, and **5o**) showed consistently excellent AI activity (≥70% inhibition) 3 and 4 h after the carrageenan injection comparable to that of standard drug indomethacin (78%), whereas the remaining twelve compounds have shown significant activity with 57–75% inhibition after 3 h and 56–63% inhibition after 4 h.

In general, compounds containing a halogen substituent showed better activity as compared to non-halogen-containing

Table 1 In vivo anti-inflammatory activity of compounds **3a–e** and **5a–o**

Compound[a]	Volume of edema (ml)[b] and %AI[c]			
	1 (h)	2 (h)	3 (h)	4 (h)
Control	0.53 ± 0.01	2.18 ± 0.09	2.20 ± 0.07	2.30 ± 0.03
Indomethacin	0.31 ± 0.02** (41)[c]	0.45 ± 0.07** (79)	0.48 ± 0.09** (78)	0.50 ± 0.05** (78)
3a	0.34 ± 0.08** (35)	0.88 ± 0.06** (59)	0.71 ± 0.09** (67)	0.84 ± 0.05** (63)
3b	0.40 ± 0.03** (24)	0.53 ± 0.08** (75)	0.58 ± 0.07** (73)	0.57 ± 0.05** (75)
3c	0.35 ± 0.04** (33)	0.51 ± 0.08** (76)	0.50 ± 0.03** (77)	0.66 ± 0.01** (71)
3d	0.36 ± 0.06** (32)	0.58 ± 0.07** (72)	0.55 ± 0.07** (75)	0.68 ± 0.01** (70)
3e	0.38 ± 0.06** (28)	0.52 ± 0.03** (76)	0.89 ± 0.08** (59)	0.66 ± 0.06** (71)
5a	0.35 ± 0.06** (33)	0.62 ± 0.07** (71)	0.67 ± 0.08** (69)	0.64 ± 0.01** (72)
5b	0.36 ± 0.04** (32)	0.58 ± 0.07** (72)	0.55 ± 0.07** (75)	0.68 ± 0.01** (70)
5c	0.38 ± 0.03** (28)	0.75 ± 0.10** (65)	0.95 ± 0.10** (57)	0.92 ± 0.05** (60)
5d	0.47 ± 0.01* (11)	0.87 ± 0.06** (60)	0.76 ± 0.04** (65)	1.00 ± 0.04* (56)
5e	0.34 ± 0.01** (35)	1.53 ± 0.02** (29)	0.58 ± 0.07** (73)	0.57 ± 0.06** (75)
5f	0.35 ± 0.05** (33)	0.59 ± 0.03** (72)	0.58 ± 0.07** (73)	0.67 ± 0.06** (70)
5g	0.41 ± 0.03** (22)	0.81 ± 0.06** (62)	0.93 ± 0.12** (57)	1.00 ± 0.10** (56)
5h	0.38 ± 0.06** (28)	0.70 ± 0.10** (67)	0.58 ± 0.07** (73)	0.95 ± 0.08** (58)
5i	0.50 ± 0.03 (5)	0.83 ± 0.03** (61)	0.66 ± 0.05** (70)	0.60 ± 0.07** (73)
5j	0.35 ± 0.03** (33)	0.87 ± 0.06** (60)	0.56 ± 0.04** (75)	1.00 ± 0.14* (56)
5k	0.36 ± 0.01** (32)	0.86 ± 0.05** (60)	0.86 ± 0.06** (60)	0.97 ± 0.02* (57)
5l	0.40 ± 0.09** (24)	0.58 ± 0.07** (72)	0.56 ± 0.04** (75)	0.88 ± 0.06** (61)
5m	0.36 ± 0.02** (32)	0.73 ± 0.05** (65)	0.87 ± 0.06** (61)	0.88 ± 0.04** (61)
5n	0.36 ± 0.05** (32)	0.72 ± 0.06** (66)	0.67 ± 0.03** (69)	0.67 ± 0.06** (71)
5o	0.33 ± 0.06** (37)	0.75 ± 0.08** (65)	0.61 ± 0.07** (72)	0.68 ± 0.03** (70)

[a] Dose levels: test compounds (50 mg/kg body wt.), indomethacin (10 mg/kg body wt.)

[b] Values are expressed as mean ± SEM and analyzed by ANOVA

[c] Values in parentheses (percentage anti-inflammatory activity, AI%)

*Significantly different compared to respective control values, $P < 0.05$

**Significantly different compared to respective control values, $P < 0.01$

compounds. For instance, six of the eight compounds containing chlorine (Cl) as one of the substituents (**3c**, **5b**, **5e**, **5h**, **5i**, and **5n**) showed excellent activity (≥70% inhibition) when measured 3 h after the carrageenan injection. The best compound in each series (**3b**, **5e**) in terms of AI activity after 4 h contains a fluoro (F) substituent at position-4 of the phenyl ring that is attached to the C-3 of the pyrazole moiety. In general, thiosemicarbazones **3a–e** showed better AI activity as compared to the thiazoles derived from them. Thus, incorporation of thioamide part of the thiosemicarbazones **3a–e** into a thiazole nucleus (**5a–o**) neither has a beneficial effect nor a detrimental effect on the AI activity. Four (**3b–d**) of the five thiosemicarbazones (**3a–e**) showed excellent AI activity comparable to that of the standard drug indomethacin. Out of four compounds (**3e**, and 5m–**o**) containing a methoxy substituent, three compounds (**3e**, **5n**, and **5o**) showed excellent AI activity (≥70% inhibition) after 4 h. These results are in accordance with our earlier observations (Sharma and Sawhney, 1997; Sharma *et al.*, 1998) claiming that the compounds with Cl (or F) and methoxy substituents show higher activity. Consistently, excellent AI activity up to 4 h suggests that these compounds do not get easily metabolized in the system.

In vitro antibacterial activity

All the target compounds were evaluated for their in vitro antibacterial activity using agar well diffusion method (Ahmad and Beg, 2001) against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) representing Gram-positive bacteria, and *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) representing Gram-negative bacteria (Table 2). Ciprofloxacin was used as the reference drug. Antibacterial activity, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8 mm. MIC of various compounds against bacterial strains was tested through a macrodilution tube method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) (Andrews, 2001) (Table 2).

Results revealed that all tested compounds **3a–3e** and **5a–5o** possessed moderate to good antibacterial activity against Gram-positive bacteria (*S. aureus*, *B. subtilis*) (Table 2). However, none of the compounds showed activity against Gram-negative bacteria (*E. coli* and

Table 2 *In vitro* antibacterial activity of compounds **3a–e** and **5a–o**

Compound[a]	Diameter of growth of inhibition zone (mm)[b]				Minimum inhibitory concentration (MIC) (μg/ml)	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Ciprofloxacin	26.3	25.6	25.0	23.3	5	5
3a	17.0	19.6	–	–	128	64
3b	15.6	15.3	–	–	>128	>128
3c	14.0	13.6	–	–	>128	>128
3d	16.3	14.3	–	–	>128	>128
3e	13.6	15.0	–	–	>128	>128
5a	14.3	14.6	–	–	>128	>128
5b	13.6	15.3	–	–	>128	>128
5c	15.0	14.6	–	–	>128	>128
5d	14.6	16.6	–	–	>128	>128
5e	14.3	13.3	–	–	>128	>128
5f	15.6	14.3	–	–	>128	>128
5g	13.3	15.6	–	–	>128	>128
5h	14.6	15.0	–	–	>128	>128
5i	15.3	14.6	–	–	>128	>128
5j	15.3	13.6	–	–	>128	>128
5k	14.6	15.3	–	–	>128	>128
5l	13.6	14.0	–	–	>128	>128
5m	15.3	15.6	–	–	>128	>128
5n	13.6	14.3	–	–	>128	>128
5o	20.6	17.3	–	–	64	128

– No activity

[a] Concentration 4.0 mg/ml

[b] Values, including diameter of the well (8 mm), are means of three replicates

P. aeruginosa). On the basis of zone of inhibition against the test bacterium, compounds **5o** and **3a** were found to be the most active molecules and showed good antibacterial activity against Gram-positive bacteria (*S. aureus*, *B. subtilis*). When compared with standard drug ciprofloxacin which showed the zone of inhibition 26.3 mm against *S. aureus* and 25.6 mm against *B. subtilis*, **3a** was found to be most effective against *B. subtilis* with zone of inhibition 19.6 mm and **5o** was found to be most effective against *S. aureus* with zone of inhibition 20.6 mm (Table 2).

Conclusion

Twenty new compounds including five thiosemicarbazones (**3a**–**e**) and fifteen thiazolylhydrazinomethylidenepyrazoles (**5a**–**o**) were synthesized and evaluated for their in vivo anti-inflammatory activity and in vitro antibacterial activity. In general thiosemicarbazones (**3a**–**e**) showed better AI activity as compared to the thiazolylhydrazinomethylidenepyrazoles (**5a**–**o**) derived from them indicating that the thiazole ring, derived from thiocarboxamide part of thiosemicarbazones (**3a**–**e**), is capable of retaining the AI activity but not advantageous. Four (**3b**–**e**) of the five thiosemicarbazones and five of the fifteen thiazole containing pyrazole derivatives (**5b**, **5e**, **5f**, **5i**, and **5o**) showed excellent AI activity ($\geq$70% inhibition) 3 h as well as 4 h after the carrageenan injection that is comparable to the standard drug indomethacin. However, none of the compounds was found to be superior over the reference drug. Though the tested compounds failed to give encouraging results in terms of their antibacterial properties, the AI activity results are promising and demand further investigations in this area.

Experimental protocols

Melting points were determined in open capillaries in electrical apparatus and are uncorrected. IR spectra were recorded on a Buck Scientific IR M500 instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75.5 MHz, respectively. The δ values are given in ppm relative to tetramethylsilane as internal standard (for ^{1}H and ^{13}C NMR). Mass spectra (DART-MS) were recorded on a JEOL-AccuTOF JMS-T100LC Mass spectrometer having a DART (direct analysis in real time) source in ES^{+} mode. Exchangeable (ex) protons were detected by disappearance of peaks upon D_2O addition. The purity of the compounds was checked by ^{1}H NMR. Iodine or UV lamp was used as a visualizing agent for thin layer chromatography (TLC).

General procedure for the synthesis of thiosemicarbazones (**3a**–**k**)

To a solution of 4-formylpyrazole **1** (1.0 mmol) in ethanol (10 ml), was added thiosemicarbazide (**2**, 1.0 mmol) followed by 4–5 drops of glacial acetic acid. The resulting reaction mixture was refluxed for 1 h, cooled to room temperature, whereupon a solid material separated out that was filtered, washed with water followed by ethanol, and dried to afford the target thiosemicarbazones **3** as solid material in excellent yield.

*2-({1-[4-(Aminosulfonyl)phenyl]-3-phenyl-1H-pyrazol-4-yl}methylidene)-1-hydrazinecarbothioamide **3a***

M.p. 196–198°C, yield 90%; IR (KBr) cm^{-1}: 3464, 3317 and 3124 (N–H stretch), 1597 (C=N stretch), 1543 (C=N stretch), 1504 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, ex, 1H, NH/SH/=NH), 9.30 (s, 1H, CH=N), 8.33 (s, ex, 1H, NH/SH/=NH), 8.23 (s, 1H, pyrazole–H), 8.09 (d, 2H, J = 8.7 Hz, Ar), 8.01 (d, 2H, J = 8.7 Hz, Ar), 7.81 (s, ex, 1H, NH/SH/=NH), 7.69 (m, 2H, Ar), 7.52 (m, 3H, Ar), 7.48 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.1, 152.5, 142.4, 141.5, 135.1, 132.2, 129.2, 128.6, 127.9, 118.9, 118.4; *m/z* 401 ($[M+H]^+$, $C_{17}H_{16}N_6O_2S_2H^+$ calcd. 401).

*2-({1-[4-(Aminosulfonyl)phenyl]-3-(4-fluorophenyl)-1H-pyrazol-4-yl}methylidene)-1-hydrazinecarbothioamide **3b***

M.p. 220–222°C, yield 94%; IR (KBr) cm^{-1}: 3456, 3333 and 3163 (N–H stretch), 1597 (C=N stretch), 1543 (C=N stretch), 1512 (N–H bend), 1342 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.43 (s, ex, 1H, NH/SH/=NH), 9.27 (s, 1H, CH=N), 8.31 (s, ex, 1H, NH/SH/=NH), 8.19 (s, 1H, pyrazole–H), 8.08 (d, 2H, J = 9.0 Hz, Ar), 7.99 (d, 2H, J = 9.0 Hz, Ar), 7.77 (s, ex, 1H, NH/SH/=NH), 7.74 (dd, $^{3}J_{HH}$ = 8.4 Hz, $^{4}J_{HF}$ = 5.4 Hz, 2H, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.38 (t, 2H, J = 8.7 Hz, Ar); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.0, 162.8 (d, $^{1}J_{CF}$ = 246.0 Hz), 151.6, 142.4, 141.4, 135.0, 130.7 (d, $^{3}J_{CF}$ = 8.3 Hz), 128.6, 127.9, 118.9, 118.3, 116.2 (d, $^{2}J_{CF}$ = 21.1 Hz); *m/z* 419 ($[M+H]^+$, $C_{17}H_{15}FN_6O_2S_2H^+$ calcd. 419).

*2-({1-[4-(Aminosulfonyl)phenyl]-3-(4-chlorophenyl)-1H-pyrazol-4-yl}methylidene)-1-hydrazinecarbothioamide **3c***

M.p. 230–231°C, yield 91%; IR (KBr) cm^{-1}: 3464, 3348 and 3163 (N–H stretch), 1597 (C=N stretch), 1543 (C=N stretch), 1504 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.43 (s, ex, 1H, NH/

SH/=NH), 9.27 (s, 1H, CH=N), 8.30 (s, ex, 1H, NH/SH/=NH), 8.20 (s, 1H, pyrazole–H), 8.09 (d, 2H, $J = 8.7$ Hz, Ar), 8.01 (d, 2H, $J = 8.7$ Hz, Ar), 7.77 (s, ex, 1H, NH/SH/=NH), 7.72 (d, 2H, $J = 8.4$ Hz, Ar), 7.58 (d, 2H, $J = 8.4$ Hz, Ar), 7.48 (s, ex, 2H, SO_2NH_2); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.1, 151.2, 142.5, 141.2, 134.9, 134.1, 131.0, 130.3, 129.2, 128.8, 127.9, 119.0, 118.4; *m/z* 435 ($[M+H]^+$, $C_{17}H_{15}ClN_6O_2S_2H^+$ calcd. 435).

2-({1-[4-(Aminosulfonyl)phenyl]-3-(4-methylphenyl)-1H-pyrazol-4-yl}methylidene)-1-hydrazinecarbothioamide ***3d***

M.p. 218-220°C, yield 95%; IR (KBr) cm^{-1}: 3464, 3348 and 3171 (N–H stretch), 1589 (C=N stretch), 1543 (C=N stretch), 1504 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.40 (s, ex, 1H, NH/SH/=NH), 9.27 (s, 1H, CH=N), 8.32 (s, ex, 1H, NH/SH/=NH), 8.21 (s, 1H, pyrazole–H), 8.07 (d, 2H, $J = 8.7$ Hz, Ar), 8.01 (d, 2H, $J = 8.7$ Hz, Ar), 7.82 (s, ex, 1H, NH/SH/=NH), 7.58 (d, 2H, $J = 8.4$ Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.32 (d, 2H, $J = 8.4$ Hz, Ar), 2.37 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.0, 152.5, 142.3, 141.5, 138.8, 135.2, 129.8, 129.3, 128.5, 127.9, 118.8, 118.3, 21.3 (CH_3); *m/z* 415 ($[M+H]^+$, $C_{18}H_{18}N_6O_2S_2H^+$ calcd. 415).

2-({1-[4-(Aminosulfonyl)phenyl]-3-(4-methoxyphenyl)-1H-pyrazol-4-yl}methylidene)-1-hydrazinecarbothioamide ***3e***

M.p. 221-223°C, yield 89%; IR (KBr) cm^{-1}: 3458, 3333 and 3171 (N–H stretch), 1597 (C=N stretch), 1543 (C=N stretch), 1506 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.42 (s, ex, 1H, NH/SH/=NH), 9.22 (s, 1H, CH=N), 8.26 (s, ex, 1H, NH/SH/=NH), 8.20 (s, 1H, pyrazole–H), 8.08 (d, 2H, $J = 8.7$ Hz, Ar), 7.99 (d, 2H, $J = 8.7$ Hz, Ar), 7.77 (s, ex, 1H, NH/SH/=NH), 7.62 (d, 2H, $J = 8.4$ Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2), 7.07 (d, 2H, $J = 8.4$ Hz, Ar), 3.82 (s, 3H, OCH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.1, 160.2, 152.4, 142.3, 141.5, 135.4, 130.0, 128.4, 127.8, 124.5, 118.8, 118.1, 114.6, 55.7 (OCH_3); *m/z* 431 ($[M+H]^+$, $C_{18}H_{18}N_6O_2S_2H^+$ calcd. 431).

General procedure for the preparation of thiazolylhydrazinomethylidenepyrazoles (**5a–o**)

To a solution of thiosemicarbazone (**3**, 1.0 mmol) in THF:EtOH (80 ml), was added α-bromoketone (**4**, 1.0 mmol) followed by a sodium acetate (1.0 mmol). The resulting reaction mixture was refluxed for 6 h, cooled to room temperature, whereupon a solid material was separated out, which was filtered to afford crude material that was crystallized from ethanol, to yield the target thiazolylhydrazinomethylidenepyrazoles **5** as solid material.

4-(3-Phenyl-4-{[2-(4-phenyl-1,3-thiazol-2-yl)hydrazono]methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5a***

M.p. 230–232°C, yield 78%; IR (KBr) cm^{-1}: 3410, 3333 and 3263 (N–H stretch), 1605 (C=N stretch), 1551 (C=N stretch), 1504 (N–H bend), 1358 and 1142 (s, SO_2 stretch) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.04 (s, ex, 1H, NH), 9.02 (s, 1H, CH=N), 8.23 (s, 1H, pyrazole–H), 8.19 (d, 2H, $J = 8.7$ Hz, Ar), 8.01 (d, 2H, $J = 8.7$ Hz, Ar), 7.80–7.86 (m, 4H, Ar), 7.53–7.55 (m, 3H, Ar), 7.48 (s, ex, 2H, SO_2NH_2), 7.40-7.43 (m, 2H, Ar), 7.37–7.38 (m, 2H, Ar, thiazole–H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.3, 152.0, 150.8, 142.3, 141.5, 135.1, 134.7, 132.4, 129.1, 129.0, 128.4, 127.9, 127.8, 125.9, 119.1, 118.2, 103.9; DART MS *m/z* 501.1160 $[M+H]^+$, $C_{25}H_{20}N_6O_2S_2H^+$ calcd. 501.1162.

4-(4-{[2-(4-(4-Chlorophenyl)-1,3-thiazol-2-yl)hydrazono]methyl}-3-phenyl-1H-pyrazol-1-yl)benzenesulfonamide ***5b***

M.p. 228–230°C, yield 74%; IR (KBr) cm^{-1}: 3456, 3310 and 3263 (N–H stretch), 1598 (C=N stretch), 1566 (C=N stretch), 1504 (N–H bend), 1335 and 1149 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.04 (s, ex, 1H, NH), 9.02 (s, 1H, CH=N), 8.22 (s, 1H, pyrazole–H), 8.18 (d, 2H, $J = 8.7$ Hz, Ar), 7.98 (d, 2H, $J = 8.7$ Hz, Ar), 7.85 (d, 2H, $J = 8.7$ Hz, Ar), 7.79 (d, 2H, $J = 8.7$ Hz, Ar), 7.44–7.55 (m, 5H, Ar, SO_2NH_2), 7.37 (s, 1H, thiazole–H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.5, 152.0, 149.6, 142.3, 141.5, 134.9, 134.0, 132.4, 129.08. 129.00, 128.5, 127.7, 127.6, 119.1, 118.1, 104.7; DART MS *m/z* 535.07425 $[M+H]^+$, $C_{25}H_{19}ClN_6O_2S_2H^+$ calcd. 535.0772.

4-(4-{[2-(4-(4-Methylphenyl)-1,3-thiazol-2-yl)hydrazono]methyl}-3-phenyl-1H-pyrazol-1-yl)benzenesulfonamide ***5c***

M.p. 185–186°C, yield 80%; IR (KBr) cm^{-1}: 3425, 3271 and 3143 (N–H stretch), 1589 (C=N stretch), 1558 (C=N stretch), 1497 (N–H bend) 1335 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.99 (bs, ex, 1H, NH), 9.01 (s, 1H, CH=N), 8.23 (s, 1H, pyrazole–H), 8.18 (d, 2H, $J = 8.7$ Hz, Ar), 7.99 (d, 2H, $J = 8.7$ Hz, Ar), 7.81 (d, 2H, $J = 8.7$ Hz, Ar), 7.73 (d, 2H, $J = 8.7$ Hz, Ar), 7.48–7.55 (m, 5H, Ar, SO_2NH_2), 7.19–7.22 (m, 3H, thiazole–H, Ar), 2.31 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.2, 152.0, 142.3, 141.5, 137.2, 134.6, 132.5, 132.4, 129.6, 129.0, 128.9, 128.4, 127.7, 125.9, 119.1, 118.2,

103.0, 21.2 (CH_3); DART MS *m/z* 515.1341 $[M+H]^+$, $C_{26}H_{22}N_6O_2S_2H^+$ calcd. 515.1318.

4-(3-(4-Fluorophenyl)-4-{[2-(4-phenyl-1,3-thiazol-2-yl)hydrazono]-methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5d***

M.p. 235–236°C, yield 71%; IR (KBr) cm^{-1}: 3456, 3354 and 3263 (N–H stretch), 1597 (C=N stretch), 1551 (C=N stretch), 1504 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6): δ 12.05 (s, ex, 1H, NH), 9.02 (s, 1H, CH=N), 8.21 (s, 1H, pyrazole–H), 8.17 (d, 2H, $J = 8.7$ Hz, Ar), 7.99 (d, 2H, $J = 8.7$ Hz, Ar), 7.84–7.86 (m, 4H, Ar), 7.50 (s, ex, 2H, SO_2NH_2), 7.38–7.40 (m, 5H, Ar), 7.30 (s, 1H, thiazole–H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.3, 162.8 (d, $^1J_{CF} = 246.1$ Hz), 150.9, 142.4, 141.4, 135.1, 134.6, 131.2 (d, $^3J_{CF} = 8.3$ Hz), 129.0, 127.9, 127.8, 125.9, 119.0, 118.1, 115.9 (d, $^2J_{CF} = 21.1$ Hz), 103.9; DART MS *m/z* 519.1096 $[M+H]^+$, $C_{25}H_{19}FN_6O_2S_2H^+$ calcd. 519.1068.

4-(4-{[2-(4-(4-Chlorophenyl)-1,3-thiazol-2-yl)-hydrazono]methyl-3-(4-fluorophenyl)}-1H-pyrazol-1-yl)benzenesulfonamide ***5e***

M.p. 230–231°C, yield 79%; IR (KBr) cm^{-1}: 3265, 3144 and 3054 (N–H stretch), 1595 (C=N stretch), 1553 (C=N stretch), 1512 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6): δ 12.02 (s, ex, 1H, NH), 9.01 (s, 1H, CH), 8.19 (s, 1H, CH), 8.16 (d, 2H, $J = 8.7$ Hz, Ar), 7.98 (d, 2H, $J = 8.7$ Hz, Ar), 7.84–7.86 (m, 4H, Ar), 7.52 (d, 2H, $J = 8.7$ Hz, Ar), 7.48 (s, ex, 2H, SO_2NH_2), 7.36 (m, 3H, thiazole–H, Ar); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.5, 162.8 (d, $^1J_{CF} = 246.8$ Hz), 150.9, 149.6, 142.4, 141.4, 134.7, 134.0, 132.3, 131.2 (d, $^3J_{CF} = 8.3$ Hz), 129.0, 127.8, 127.6, 119.0, 118.0, 115.9 (d, $^2J_{CF} = 21.8$ Hz), 104.7; DART MS *m/z* 553.0702 $[M+H]^+$, $C_{25}H_{18}ClFN_6O_2S_2H^+$ calcd. 553.0678.

4-(3-(4-Fluorophenyl)-4-{[2-(4-(4-methylphenyl)-1,3-thiazol-2-yl)-hydrazono]methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5f***

M.p. 233–235°C, yield 83%; IR (KBr) cm^{-1}: 3253, 3154 and 3063 (N–H stretch), 1595 (C=N stretch), 1553 (C=N stretch), 1504 (N–H bend), 1331 and 1157 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6); δ 12.05 (s, ex, 1H, NH), 9.02 (s, 1H, CH=N), 8.20 (s, 1H, Pyrazole–H), 8.15 (d, 2H, $J = 8.7$ Hz, Ar), 7.97 (d, 2H, $J = 8.7$ Hz, Ar), 7.85 (dd, 2H, $^3J_{HH} = 8.4$ Hz, $^4J_{HF} = 5.4$ Hz, Ar), 7.72 (d, 2H, $J = 8.4$ Hz, Ar), 7.34 (t, 2H, $J = 8.4$ Hz, Ar), 7.47 (s, ex, 2H, SO_2NH_2), 7.19–7.22 (m, 3H, thiazole–H, Ar), 2.31 (s, 3H,CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.2, 162.6 (d, $^1J_{CF} = 246.6$ Hz), 150.9, 142.3, 141.4, 137.2, 131.1 (d, $^3J_{CF} = 8.3$ Hz), 129.0, 127.8, 125.9, 119.0, 118.1, 115.9 (d, $^2J_{CF} = 21.9$ Hz), 103.0, 21.2 (CH_3); DART MS *m/z* 533.1402 $[M+H]^+$, $C_{26}H_{21}FN_6O_2S_2H^+$ calcd. 533.1224.

4-(3-(4-Chlorophenyl)-4-{[2-(4-phenyl-1,3-thiazol-2-yl)hydrazono]-methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5g***

M.p. 208–209°C, yield 81%; IR (KBr) cm^{-1}: 3448, 3379 and 3256 (N–H stretch), 1595 (C=N stretch), 1556 (C=N stretch), 1504 (N–H bend), 1335 and 1157 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6): δ 12.02 (s, ex, 1H, NH), 9.00 (s, 1H, CH=N), 8.19 (s, 1H, pyrazole–H), 8.16 (d, 2H, $J = 8.7$ Hz, Ar), 7.98 (d, 2H, $J = 8.7$ Hz, Ar), 7.87 (d, 2H, $J = 8.4$ Hz, Ar), 7.84 (d, 2H, $J = 8.4$ Hz, Ar), 7.59 (d, 2H, $J = 8.4$ Hz, Ar), 7.48 (s, ex, 2H, SO_2NH_2), 7.39 (t, 2H, $J = 8.1$ Hz, Ar), 7.29–7.31 (m, 2H, thiazole–H, Ar); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.3, 150.9, 150.6, 142.4, 141.4, 135.1, 134.5, 133.9, 131.3, 130.7, 129.3, 129.0, 127.9, 127.8, 125.9, 119.1, 118.2, 103.9.; DART MS *m/z* 535.0794 $[M+H]^+$, $C_{25}H_{19}ClN_6O_2S_2H^+$ calcd. 535.0772.

4-(3-(4-Chlorophenyl)-4-{[2-(4-(4-chlorophenyl)-1,3-thiazol-2-yl)-hydrazono]methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5h***

M.p. 216–218°C, yield 82%; IR (KBr) cm^{-1}: 3258, 3152 and 3061 (N–H stretch), 1595 (C=N stretch), 1551 (C=N stretch), 1504 (N–H bend), 1337 and1156 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH), 9.00 (s, 1H, CH=N), 8.22 (s, 1H, pyrazole–H), 8.16 (d, 2H, $J = 8.4$ Hz, Ar), 7.99 (d, 2H, $J = 8.4$ Hz, Ar), 7.85-7.87 (m, 4H, Ar), 7.59 (d, 2H, $J = 8.4$ Hz, Ar), 7.43–7.48 (m, 4H, SO_2NH_2, Ar), 7.35 (s, 1H, thiazole–H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.4, 150.6, 149.7, 142.4, 141.4, 134.7, 133.9, 132.3, 131.3, 130.7, 129.3, 129.0, 127.8, 127.6, 119.1, 118.2, 104.7; DART MS *m/z* 569.0365 $[M+H]^+$, $C_{25}H_{18}Cl_2N_6O_2S_2H^+$ calcd. 569.0382.

4-(3-(4-Chlorophenyl)-4-{[2-(4-(4-methylphenyl)-1,3-thiazol-2-yl)-hydrazono]methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5i***

M.p. 226–228°C, yield 81%; IR (KBr) cm^{-1}: 3258, 3153 and 3044 (N–H stretch), 1597 (C=N stretch), 1553 (C=N stretch), 1509 (N–H bend) 1339 and1148 (s, SO_2 stretch); ^1H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH), 9.01 (s, 1H, CH=N), 8.20 (s, 1H, pyrazole–H), 8.16 (d, 2H, $J = 8.4$ Hz, Ar), 7.99 (d, 2H, $J = 8.4$ Hz, Ar), 7.87 (d, 2H, $J = 8.4$ Hz, Ar), 7.72 (d, 2H, $J = 8.4$ Hz, Ar), 7.58 (d, 2H, $J = 8.4$ Hz, Ar), 7.51 (s, ex, 2H, SO_2NH_2), 7.17–7.20 (m, 3H, thiazole–H, Ar), 2.30 (s, 3H, CH_3); ^{13}C NMR

(75.5 MHz, DMSO-d_6): δ 168.2, 150.9, 150.6, 142.4, 141.4, 137.2, 134.4, 133.9, 132.4, 131.3, 130.7, 129.6, 129.2, 129.0, 127.8, 125.9, 119.1, 118.2, 103.0, 21.2 (CH_3); DART MS *m/z* 549.0946 $[M+H]^+$, $C_{26}H_{21}ClN_6O_2S_2H^+$ calcd. 549.0929.

4-(3-(4-Methylphenyl)-4-{[2-(4-phenyl-1,3-thiazol-2-yl)hydrazono]-methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5j***

M.p. 220–221°C, yield 82%; IR (KBr) cm^{-1}: 3256, 3153 and 3064 (N–H stretch), 1504 (N–H bend), 1327 and1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.05 (bs, ex, 1H, NH), 8.99 (s, 1H, CH=N), 8.22 (s, 1H, pyrazole–H), 8.19 (d, 2H, $J = 8.7$ Hz, Ar), 7.99 (d, 2H, $J = 8.7$ Hz, Ar), 7.86 (d, 2H, $J = 8.7$ Hz, Ar), 7.71 (d, 2H, $J = 8.7$ Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.31–7.43 (m, 3H, thiazole–H, Ar), 2.39 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.3, 152.1, 150.8, 142.3, 141.5, 138.6, 135.1, 134.9, 129.6, 129.5, 129.0, 128.8, 128.2, 127.9, 127.8, 126.0, 119.0, 118.1, 103.9, 21.4 (CH_3); DART MS *m/z* 515.1336 $[M+H]^+$, $C_{26}H_{22}N_6O_2S_2H^+$ calcd. 515.1318.

4-(4-{[2-(4-(4-Chlorophenyl)-1,3-thiazol-2-yl)hydrazono]methyl}-3-(4-methylphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***5k***

M.p. 230–232°C, yield 73%; IR (KBr) cm^{-1}: 3256, 3152 and 3061 (N–H stretch), 1597 (C=N stretch), 1556 (C=N stretch), 1503 (N–H bend) 1341 and 1153 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH), 8.99 (s, 1H, CH=N), 8.21 (s, 1H, pyrazole–H), 8.17 (d, 2H, $J = 8.4$ Hz, Ar), 7.98 (d, 2H, $J = 8.4$ Hz, Ar), 7.86 (d, 2H, $J = 8.4$ Hz, Ar), 7.69 (d, 2H, $J = 8.4$ Hz, Ar), 7.44–7.47 (m, 4H, SO_2NH_2, Ar), 7.33-7.37 (m, 3H, thiazole–H, Ar), 2.40 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.1, 150.9, 150.6, 142.3, 141.2, 136.9, 134.2, 134.1, 132.1, 131.3, 130.8, 129.6, 129.1, 129.0, 127.6, 125.9, 119.1, 118.2, 103.2, 21.4 (CH_3); DART MS *m/z* 549.0954 $[M+H]^+$, $C_{26}H_{21}ClN_6O_2S_2H^+$ calcd. 549.0929.

4-(3-(4-Methylphenyl)-4-{[2-(4-(4-methylphenyl)-1,3-thiazol-2-yl)-hydrazono]methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5l***

M.p. 197–198°C, yield 78%; IR (KBr) cm^{-1}: 3263, 3152 and 3053 (N–H stretch), 1566 (C=N stretch), 1504 (N–H bend), 1335 and 1149 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH), 8.97 (s, 1H, CH=N), 8.21 (s, 1H, pyrazole–H), 8.17 (d, 2H, $J = 8.7$ Hz, Ar), 7.98 (d, 2H, $J = 8.7$ Hz, Ar), 7.73 (d, 2H, $J = 8.4$ Hz, Ar), 7.70 (d, 2H, $J = 8.4$ Hz, Ar), 7.46 (s, ex, 2H, SO_2NH_2), 7.35 (d, 2H, $J = 8.4$ Hz, Ar), 7.19–7.21 (d, 3H, thiazole–H, Ar), 2.39 (s, 3H, CH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.2, 152.0, 150.9, 142.3, 141.5, 138.6, 137.2, 134.8, 129.67, 129.62, 129.5, 128.8, 128.2, 127.9, 125.9, 119.0, 118.1, 103.0, 21.3 (CH_3), 21.2 (CH_3); DART MS *m/z* 529.1441 $[M+H]^+$, $C_{27}H_{25}N_6O_2S_2H^+$ calcd. 529.1475.

4-(3-(4-Methoxyphenyl)-4-{[2-(4-phenyl-1,3-thiazol-2-yl)hydrazono]-methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5m***

M.p. 233–234°C, yield 84%; IR (KBr) cm^{-1}: 3263, 3154 and 3063 (N–H stretch), 1574 (C=N stretch), 1512 (N–H bend), 1333 and 1157 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH), 8.98 (s, 1H, CH=N), 8.22 (s, 1H, pyrazole–H), 8.18 (d, 2H, $J = 8.4$ Hz, Ar), 7.99 (d, 2H, $J = 8.4$ Hz, Ar), 7.86 (d, 2H, $J = 8.4$ Hz, Ar), 7.75 (d, 2H, $J = 8.4$ Hz, Ar), 7.49 (s, ex, 2H, SO_2NH_2), 7.40 (t, 2H, $J = 8.4$ Hz, Ar), 7.29–7.31 (m, 2H, thiazole–H, Ar), 7.09 (d, 2H, $J = 8.4$ Hz, Ar), 3.84 (s, 3H, OCH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.4,160.1, 151.9, 150.9, 142.2, 141.5, 135.1, 134.9, 130.3, 129.0, 128.3, 127.9, 127.7, 125.9, 124.7, 119.0, 117.9, 114.5, 103.9, 55.7 (OCH_3); DART MS *m/z* 531.1287 $[M+H]^+$, $C_{26}H_{22}N_6O_2S_2H^+$ calcd. 531.1268.

4-(4-{[2-(4-(4-Chlorophenyl)-1,3-thiazol-2-yl)-hydrazono]methyl}-3-(4-methoxy-phenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***5n***

M.p. 168–170°C, yield 74%; IR (KBr) cm^{-1}: 3243, 3134 and 3061 (N–H stretch), 1597 (C=N stretch), 1551 (C=N stretch), 1504 (N–H bend), 1337 and 1154 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.02 (s, ex, 1H, NH), 8.98 (s, 1H, CH=N), 8.30 (s, 1H, pyrazole–H), 8.17 (d, 2H, $J = 8.4$ Hz, Ar), 7.97 (d, 2H, $J = 8.4$ Hz, Ar), 7.86 (d, 2H, $J = 8.4$ Hz, Ar), 7.67 (d, 2H, $J = 8.4$ Hz, Ar), 7.44–7.47 (m, 4H, SO_2NH_2, Ar), 7.38 (s, 1H, thiazole–H), 7.09 (d, 2H, $J = 8.4$ Hz, Ar), 3.84 (s, 3H, OCH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.5, 160.1, 151.9, 149.6, 142.2, 141.5, 135.1, 134.0, 132.3, 130.3, 129.0, 128.3, 127.7, 127.6, 124.7, 119.0, 117.9, 114.5, 104.7, 55.7 (OCH_3); DART MS *m/z* 565.0906 $[M+H]^+$, $C_{25}H_{19}ClN_6O_2S_2H^+$ calcd. 565.0878.

4-(3-(4-Methoxyphenyl)-4-{[2-(4-(4-methylphenyl)-1,3-thiazol-2-yl)-hydrazono]-methyl}-1H-pyrazol-1-yl)benzenesulfonamide ***5o***

M.p. 170–171°C, yield 80%; IR (KBr) cm^{-1}: 3261, 3153 and 3033 (N–H stretch), 1596 (C=N stretch), 1553 (C=N stretch), 1511 (N–H bend), 1333 and 1156 (s, SO_2 stretch); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.03 (s, ex, 1H, NH),

8.96 (s, 1H, CH=N), 8.28 (s, 1H, pyrazole–H), 8.18 (d, 2H, $J = 8.4$ Hz, Ar), 7.99 (d, 2H, $J = 8.4$ Hz, Ar), 7.83 (d, 2H, $J = 8.4$ Hz, Ar), 7.66 (d, 2H, $J = 8.4$ Hz, Ar), 7.47 (s, ex, 2H, SO_2NH_2), 7.38 (d, 2H, $J = 8.4$ Hz, Ar), 7.26 (s, 1H, thiazole–H), 7.08 (d, 2H, $J = 8.4$ Hz, Ar), 3.79 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.2, 160.5, 153.8, 142.9, 142.1, 141.5, 134.2, 132.4, 130.1, 129.2, 128.4, 127.8, 127.6, 123.9, 119.0, 117.1, 114.5, 104.4, 55.7 (OCH_3), 21.8 (CH_3); DART MS m/z 545.1456 $[M+H]^+$, $C_{27}H_{25}ClN_6O_3S_2H^+$ calcd. 545.1424.

Pharmacological assay

Carrageenan-induced rat paw edema assay

Male Wistar albino rats weighing 200–250 g were used throughout the study. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. Food was withdrawn 12 h before and during experimental hours. The animals were randomly divided into groups each consisting of six rats. One group of six rats was kept as control and received tween 80 (95:5). Another group received the standard drug indomethacin at a dose of 10 mg/kg body weight ip. Other groups of rats were administered the test compounds at a dose of 50 mg/kg body weight orally. A mark was made on the left hind paw just beyond the tibiotarsal articulation, so that, every time the paw was dipped up to the fixed mark, a constant paw volume was ensured. Paw volumes were measured using a plethysmometer (model 7140, Ugo Basile, Italy). Thirty minutes after administration of test compounds and standard drug, 0.1 ml of 1% w/v of carrageenan suspension in normal saline was injected into subplanter region of the left hind paw of all the animals. The initial paw volume was measured within 30 s of the injection and remeasured again 1, 2, 3, and 4 h after administration of carrageenan. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follows:

$$\%\text{Inhibition} = (V_t - V_0)\text{control} - (V_t - V_0)\text{tested compound}/(V_t - V_0)\text{control} \times 100$$

where V_t = volume of edema at specific time interval and V_0 = volume of edema at zero time interval.

In vitro antibacterial assay

The antibacterial activity of newly synthesized compounds was evaluated in vitro by agar–well diffusion method. (Ahmad and Beg, 2001) All the microbial cultures were adjusted to 0.5 McFarland standards, which are visually comparable to a microbial suspension of $\sim 1.5 \times 10^8$ cfu/ml (McFaraland, 1907). 20 ml of Mueller–Hinton agar media was poured into each Petri plate, and plates were swabbed with 100 µl inocula of the test microorganisms, and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates, and these were loaded with a 100 µl volume with concentration of 4 mg/ml of each compound reconstituted in the dimethylsulfoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antibacterial activity of twenty synthetic compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard. Minimum inhibitory concentration (MIC) of the newly synthesized compounds against tested bacteria was determined using macrodilution tube method as recommended by NCCLS (2000).

Acknowledgments The authors (P. K. and N. C.) are grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for the awards of junior and senior research fellowships, respectively. The authors are thankful to the Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Lucknow for the Mass spectra facilities.

References

Ahmad I, Beg AJ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 74:13–123

Anderson DW, Argentien DC, Ritchie DM, Katz LB, Shriver DA, Rosenthale ME, Capetola RJ (1990) Gastrointestinal (GI) profile of tepoxalin (TX), an orally active dual cyclooxygenase (CO)/lipoxygenase (LO) inhibitor with potent antiinflammatory activity. FASEB J 4:A1122

Andrews JM (2001) Determination of minimum inhibitory concentrations. Antimicrob Chemother 48:5–16

Banoglu E, Akoglu Ç, Unlu S, Kupeli E, Yesilada E, Sahin MF (2004) Amide derivatives of [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acids as potential analgesic and anti-inflammatory compounds. Arch Pharm Med Chem 337:7–14

Bekhit AA, Abdel-Azeim T (2004) Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory–antimicrobial agents. Bioorg Med Chem 12:1935–1945

Bekhit AA, Fahmy HTY (2003) Design and synthesis of some substituted 1*H*-pyrazolyl-oxazolidines or 1*H*-pyrazolyl-thiazolidines as anti-inflammatory-antimicrobial agents. Arch Pharm Med Chem 2:111–118

Bekhit AA, Fahmi HTY, Rostom SAF, Baraka AM (2003) Design and synthesis of some substituted 1H-pyrazolyl-thiazolo[4, 5-

d]pyrimidines as anti-inflammatory–antimicrobial agents. Eur J Med Chem 38:27–36

Bekhit AA, Ashour HMA, Guemei AA (2005) Novel pyrazole derivatives as potential promising anti-inflammatory antimicrobial agents. Arch Pharm Chem Life Sci 338:167–174

Bekhit AA, Rahman HMA, Guemei AA (2006) Synthesis and biological evaluation of some hydroxypyrazole derivatives as anti-inflammatory–antimicrobial agents. Arch Pharm Chem Life Sci 339:81–87

Bekhit AA, Ashour HMA, Bekhit A, El-Din A, Abdel-Rahman HM, Bekhit SA (2009) Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents. J Enzyme Inhib Med Chem 24:296–309

Bondock S, Khalifa W, Fadda AA (2007) Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3, 4-dihydronaphthalene-2-carboxaldehyde. Eur J Med Chem 42:948–954

Dannhardt G, Keifer W (2001) Cyclooxygenase inhibitors—current status and future prospects. Eur J Med Chem 36:109–115

Foks H, Pancechowska-Ksepko D, Kedzia A, Zwolska Z, Janowiec M, Augustynowicz-Kopec E (2005) Synthesis and antibacterial activity of 1H-pyrazolo[3,4-b]pyrazine and -pyridine derivatives. II Farmaco 60:513–517

Giri RS, Thakar HM, Giordano T, Williams J, Rogers D, Sudersanam V, Vasu KK (2009) Design, synthesis and characterization of novel 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3H-quinazoline-4-one derivatives as inhibitors of NF-κB and AP-1 mediated transcription activation and as potential anti-inflammatory agents. Eur J Med Chem 44:2184–2189

Hantsch A, Weber JH (1887) Ueber verbindungen des thiazols (pyridins der thiophenreihe). Chem Ber 20:3118

Kalkhambkar RG, Kulkarni GM, ShivKumar H, Rao RN (2007) Synthesis of novel triheterocyclic thiazoles as anti-inflammatory and analgesic agents. Eur J Med Chem 42:1272–1276

Kameyama T, Nabeshima T (1978) Effects of 1,3-diphenyl-5-(2-dimethylaminopropionamide)-pyrazole[difenamizole] on a conditioned avoidance response. Neuropharmacology 17:249–256

Kameyama T, Ukai M, Nabeshima T (1978) Inhibitory effect of difenamizole on morphine induced straub tail reaction with special reference to monoamineergic agents. Chem Pharm Bull 26:3265–3270

Karegoudar P, Karthikeyan MS, Prasad DG, Mahalinga M, Holla BS, Kumari NS (2008) Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents. Eur J Med Chem 43:261–267

Kouatly O, Geronikaki A, Kamoutsis C, Hadjipavlou-Litina DEle (2009) Adamantane derivatives of thiazolyl-*N*-substituted amide, as possible non-steroidal anti-inflammatory agents. Eur J Med Chem 44:1198–1204

LS NCC (2000) Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically approved standards, 5th edn. National Committee for Clinical Laboratory Standards, Villanova

McFaraland J (1907) The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 14:1176–1178

Nussmeier NA, Whelton AA, Brown MT, Langford RM, Hoeft A, Parlow JL, Boyce SW, Verburg KM (2005) Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. N Engl J Med 352:1081–1091

Penning TD, Talley JJ, Bertneshaw SR, Carten JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, Anderson GD, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY, Isakson PC (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). J Med Chem 40:1347–1365

Pillai AD, Rathod PD, Franklin PX, Patel M, Nivsarkar M, Vasu KK, Padh H, Sudarsanam V (2003) Novel drug designing approach for dual inhibitors as anti-inflammatory agents: implication of pyridine template. Biochem Biophys Res Commun 301:183–187

Prakash O, Kumar R, Parkash V (2008) Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones. Eur J Med Chem 43:435–440

Rosati O, Curini M, Marcotullio MC, Macchiarulo A, Perfumi M, Mattioli L, Rismondo F, Cravotto G (2007) Synthesis, docking studies and anti-inflammatory activity of 4,5,6,7-tetrahydro-2H-indazole derivatives. Bioorg Med Chem 15:3463–3473

Sawhney SN, Sharma PK (1993) Synthesis and antiinflammatory activity of some 3-heterocycle-1,2-benzisothiazoles. Bioorg Med Chem Lett 3:1551–1554

Scheen AJ (2004) Withdrawal of rofecoxib (Vioxx): what about cardiovascular safety of COX-2 selective non-steroidal anti-inflammatory drugs? Rev Med Liege 59:565–569

Shamroukh AH, Zaki MEA, Morsy EMH, Abdel-Motti FM, Abdel-Megeid FME (2007) Synthesis, isomerization, and antimicrobial evaluation of some pyrazolopyranotriazolo-pyrimidine derivatives. Arch Pharm Chem Life Sci 340:345–351

Sharma PK, Sawhney SN (1993) Synthesis and antiinflammatory activity of some 1,4-dihydro-3-methyl-1-(2-thiazolyl)pyrazolo[4,3-c][1, 2]benzothiazine 5,5-dioxides. Bull Chem Soc Japan 66:3843–3846

Sharma PK, Sawhney SN (1997) Potent antiinflammatory 3-thiazole-4(5)-acetic acids of 1,2-benzisothiazole. Bioorg Med Chem Lett 7:2427–2430

Sharma PK, Sawhney SN, Gupta A, Singh GB, Bani S (1998) Synthesis and antiinflammatory activity of some 3-(2-thiazolyl)-1, 2-benzisothiazoles. Indian J Chem 37B:376–381

Sharma PK, Kumar S, Kumar P, Kaushik P, Kaushik D, Dhingra Y, Aneja KR (2010) Synthesis and biological evaluation of some pyrazolylpyrazolines as anti-inflammatory–antimicrobial agents. Eur J Med Chem 45:2650–2655

Sharma PK, Chandak N, Kumar P, Sharma C, Aneja KR (2011a) Synthesis and biological evaluation of some 4-functionalized-pyrazoles as antimicrobial agents. Eur J Med Chem 46:1425–1432

Sharma PK, Singh K, Kumar S, Kumar P, Dhawan SN, Lal S, Ulbrich H, Dannhardt G (2011b) Synthesis and anti-inflammatory evaluation of some pyrazolo[3,4-b]pyridines. Med Chem Res 20:239–244

Szabó G, Fischer J, Kis-Varga A, Gyires K (2008) New celecoxib derivatives as anti-inflammatory agents. J Med Chem 51:142–147

Venkatachalam SR, Salaskar A, Chattopadhyay A, Barik A, Mishra B, Gangabhagirathic R, Priyadarsini KI (2006) Synthesis, pulse radiolysis, and in vitro radioprotection studies of melatoninolipoamide, a novel conjugate of melatonin and α-lipoic acid. Bioorg Med Chem 14:6414–6419

Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 111:544–547