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Original article

Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1*H*-pyrazole as anti-inflammatory antimicrobial agents

Adnan A. Bekhit [a,*], Hayam M.A. Ashour [a], Yasser S. Abdel Ghany [a], Alaa El-Din A. Bekhit [b], Azza Baraka [c]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt*
[b] *Agriculture and Life Sciences Division, Lincoln University, Christchurch, New Zealand*
[c] *Department of Pharmacology, Faculty of Medicine, University of Alexandria, Alexandria 21521, Egypt*

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Abstract

Four series of pyrazolyl benzenesulfonamide derivatives have been synthesized. The first series was prepared by cyclization of the intermediate *N*,*N*-dimethylaminomethylene-4[3-phenyl-4-(substituted thiosemicarbamoyl hydrazonomethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide **2a–c** with ethyl bromoacetate to afford the corresponding thiazolidinyl derivatives **3a–c**. The second series was prepared by cyclization of the key intermediates **2a–c** with 4-bromophenacyl bromide giving rise to thiazolinyl derivatives **4a–c**. Thiadiazolyl derivatives **5a–c** were obtained by heating **2a–c** with 2 M $FeCl_3$ solution. Refluxing the intermediates **2a–c** in acetic anhydride yielded the corresponding thiadiazolinyl derivatives **6a–c**. All the target compounds showed anti-inflammatory activity and three of them **3b**, **3c** and **4c** surpassed that of indomethacin both locally and systemically in the cotton pellet granuloma and rat paw edema bioassay. The active compounds showed selective inhibitory activity towards COX-2 enzyme as revealed by the in vitro enzymatic assay. All the tested compounds proved to have superior gastrointestinal (GI) safety profiles as compared to indomethacin, when tested for their ulcerogenic effects. The acute toxicity study of compounds having promising anti-inflammatory activity (**3b**, **3c** and **4c**) indicated that they are well tolerated both orally and parenterally. Antimicrobial activity tests expressed as minimal inhibitory concentrations (MIC), revealed that compounds **3b** and **4a** showed comparable antibacterial activity to that of ampicillin against *Escherichia coli*, while compounds **3a**, **3c** and **4a** possessed about half the activity of ampicillin against *Staphylococcus aureus*. On the other hand, the results showed that all the tested compounds have weak or no antifungal activity against *Candida albicans* except for compounds **6b** and **6c** that showed half the activity of the control antifungal drug used (clotrimazole).

Keywords: Pyrazole; Anti-inflammatory; Antimicrobial; Ulcerogenic effect; Acute toxicity

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been recognized as important class of therapeutic agents for the alleviation of pain and inflammation associated with a number of pathological conditions. However, long term use of NSAIDs has been associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity [1–4]. Consequently, extensive research has been directed towards improving their pharmacological profile that led to the discovery of multiple isoforms of cyclooxygenase (COX) that are differently regulated [5,6]. The discovery of the inducible isoform of cyclooxygenase enzyme (COX-2) spurred the search for anti-inflammatory agents devoid of the undesirable effects associated with classical NSAIDs. Recently, a novel class of selective COX-2 inhibitors has been discovered. Among this class, celecoxib (Fig. 1) was shown to be a potent and gastrointestinal (GI) safe anti-inflammatory

* Corresponding author. Tel.: +20 3 4873273.
E-mail address: adnbekhit@hotmail.com (A.A. Bekhit).

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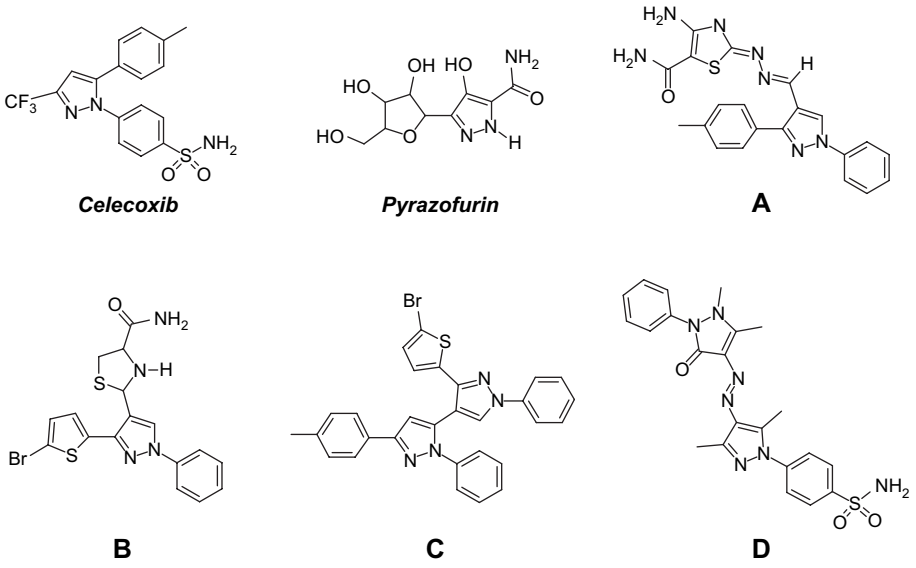


Fig. 1. Structures of celecoxib and reported active pyrazole derivatives A, B, C and D.

analgesic agent. It is considered a typical model of pyrazole containing, diaryl-heterocyclic template that is known to selectively inhibit COX-2 [7]. Furthermore, several other compounds containing pyrazole functionality were also reported to exhibit anti-inflammatory activity [8–12].

Much attention was given to pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside, pyrazofurin (Fig. 1) which demonstrated a broad spectrum antimicrobial activity [13,14]. Consequently, several pyrazole derivatives that exhibited antimicrobial activity were reported by Tanitame and coworkers [15–17].

Multi-drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Hence, mono therapy with a drug having both anti-inflammatory and antimicrobial activities is highly desirable, both from the pharmacoeconomic and patient compliance points of view, which have been the goal of our ongoing research program [18–28]. We have recently reported the anti-inflammatory and antimicrobial activities of some lead compounds containing pyrazole moiety attached to different heterocyclic ring systems [18–28]. Some of these lead compounds, A [20], B [21], C [22,23] and D [24], showed pronounced dual anti-inflammatory and antimicrobial activities.

In the present study, we report the synthesis of different novel pyrazolyl benzenesulfonamide derivatives bearing thiazolyl and thiadiazolyl ring systems. The target compounds were designed so as to incorporate some pharmacophoric assemblies in celecoxib, represented by an aminosulfonylphenyl group attached to a pyrazole ring bearing a lipophilic group, in addition to heterocyclic ring systems known to have anti-inflammatory and/or antimicrobial activities namely, thiazole and thiadiazole rings [29,30], which are substituted with additional lipophilic moieties. We also report the effects of such molecular variations on the anti-inflammatory and antimicrobial activities of pyrazole nucleus. The enhanced overall lipophilic characteristics of the target compounds could favor their selectivity towards COX-2 enzyme over COX-1. Thus, it was of interest to examine the inhibitory effects of the synthesized compounds on COX-1 and COX-2 enzymes. Furthermore, the ulcerogenic and acute toxicity profiles of the active compounds were determined.

2. Chemistry

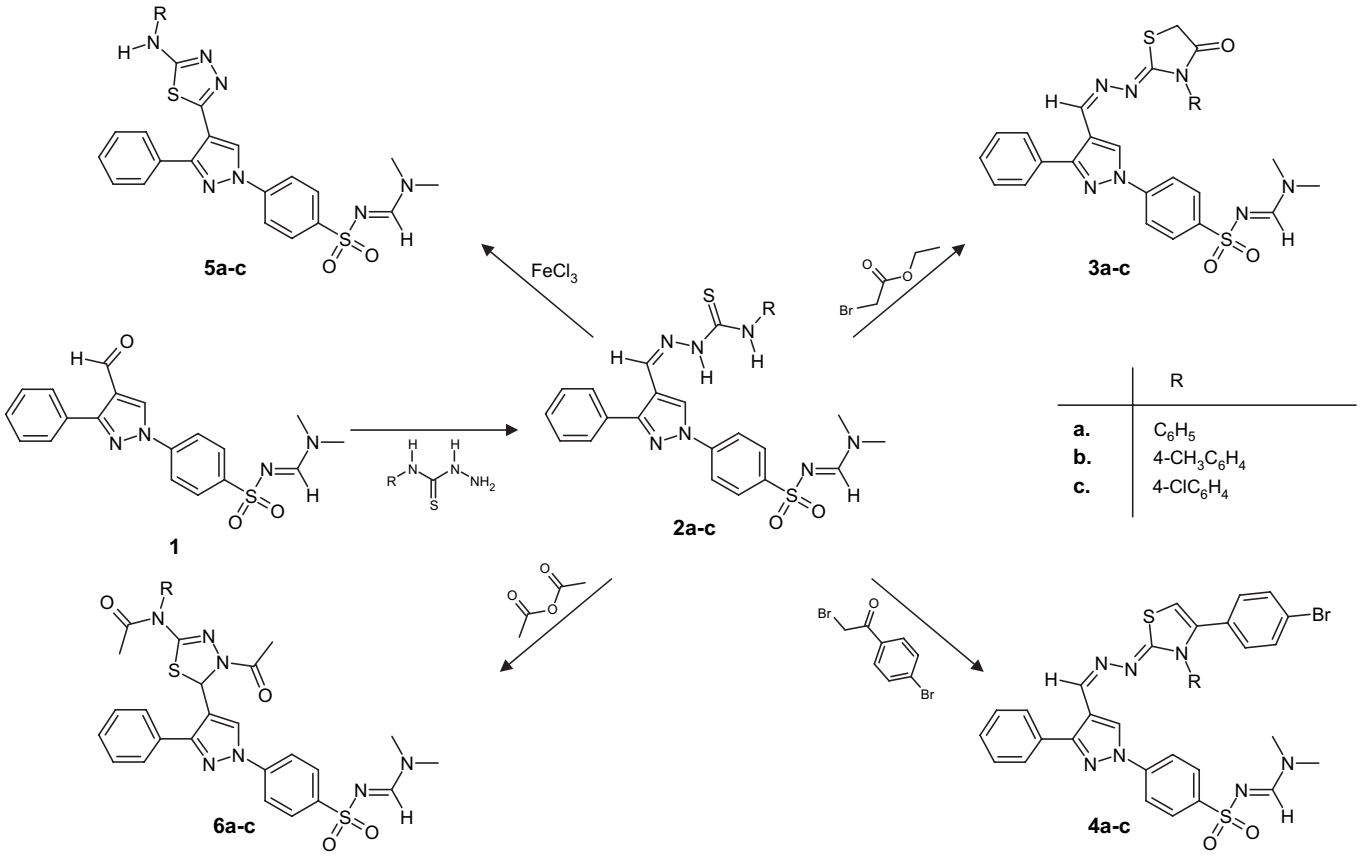
The target compounds were synthesized according to steps outlined in Scheme 1. The key intermediates *N*,*N*-dimethylaminomethylene-4-[3-phenyl-4-(substituted thiosemicarbamoylhydrazonomethyl)-1*H*-pyrazol-1-yl]benzenesulfonamides **2a–c** were obtained by condensation of *N*,*N*-dimethylaminomethylene 4-(4-formyl-3-phenyl-1*H*-pyrazol-1-yl) benzenesulfonamide **1** [28] with N^4-substituted thiosemicarbazides. Treatment of the key intermediates **2a–c** with ethyl bromoacetate produced the corresponding thiazolidinonyl derivatives **3a–c**. Similarly, reaction of **2a–c** with 4-bromophenacyl bromide yielded the corresponding thiazolinyl derivatives **4a–c**. Heating the key intermediates **2a–c** with 2 M $FeCl_3$ solution afforded the corresponding 1,3,4-thiadiazole derivatives **5a–c**. Furthermore, intermediates **2a–c** were converted to 1,3,4-thiadiazolines **6a–c** by refluxing with acetic anhydride.

3. Results and discussion

3.1. Biological evaluation

3.1.1. Anti-inflammatory activity

3.1.1.1. Cotton pellet-induced granuloma bioassay. The anti-inflammatory activity of the target compounds **3**, **4**, **5** and **6** was evaluated by applying the cotton pellet-induced granuloma bioassay in rats [31] using indomethacin as reference



Scheme 1.

standard. The ED_{50} value for each of the tested compounds was expressed as the mean ± SEM. The difference between the control and the treated groups was estimated using student's *t*-test and was considered significant when $P < 0.001$ (Table 1). All the target compounds showed significant anti-inflammatory activity comparable to the standard used, and three of them, **3b**, **3c** and **4c** possessed anti-inflammatory activity ($ED_{50} = 9.34$, 9.12 and 9.56 μmol, respectively), surpassing that of indomethacin ($ED_{50} = 9.58$ μmol) (Table 1). The results indicated that compounds having thiazolidinonyl and thiazolinyl substituents separated from the pyrazole ring by a hydrazonomethyl spacer, **3a–c** and **4a–c**, appeared to possess higher anti-inflammatory activity than pyrazoles directly linked to thiadiazolyl moieties, **5a–c** and **6a–c**. Within the active series (**3a–c** and **4a–c**), a direct association between the lipophilic character of the substituents (from phenyl to tolyl to *p*-chlorophenyl) and the anti-inflammatory activity may exist.

Table 1
The anti-inflammatory activity[a] (ED_{50}, μmol) and ulcerogenic activity[a]

Test compound	ED_{50} (μmol)	% Ulceration
Indomethacin	9.58	100
3a	13.36	10
3b	9.34	20
3c	9.12	10
4a	12.96	0.0
4b	11.06	0.0
4c	9.56	0.0
5a	11.38	0.0
5b	12.46	10
5c	11.88	10
6a	12.54	20
6b	14.86	10
6c	13.96	10

[a] All data (tested compounds and indomethacin) were significantly different from control ($P < 0.001$).

3.1.1.2. Carrageenan-induced rat paw edema bioassay. Compounds showing promising anti-inflammatory activity in the cotton pellet-induced granuloma bioassay (**3b**, **3c** and **4c**), were further evaluated for their in vivo systemic effect using carrageenan-induced paw edema bioassay in rats [32]. The results revealed that compound **3c** exhibited systemic anti-inflammatory activity (% protection = 77.5) exceeding that of indomethacin (% protection = 74.4), whereas compounds **3b** and **4c** (% protection = 73.4 and 70.4, respectively), were slightly less active than indomethacin (Table 2). The results revealed that the systemic activity of the active compounds are in perfect agreement with their local effects.

3.1.1.3. Human COX-1 and COX-2 enzymatic activity. Compounds **3b**, **3c** and **4c** were further tested for their ability to inhibit human COX-1 and COX-2 enzymes in vitro, according to Wakitani et al. [33]. COX-1 assay was carried out using human platelets microsomal fraction prepared from NSAID-free

Table 2
Effects of compounds **3b**, **3c** and **4c** on Carrageenan-induced rat paw edema (ml), percentage protection and activity relative to indomethcin

Test compound	Increase in paw edema (ml) ± SEM[a,b]	% Protection	Activity relative to indomethacin
Control	0.98 ± 0.027	0.0	0.0
Indomethacin	0.25 ± 0.024	74.4	100
3b	0.26 ± 0.0028	73.4	98.65
3c	0.22 ± 0.027	77.4	104.16
4b	0.29 ± 0.026	70.4	94.46

[a] SEM denoted the standard error of the mean.
[b] All data are significantly different from control ($P < 0.001$).

normal human volunteers as described by Hammarström and Falardeau [34]. COX-2 assay was performed using human recombinant COX-2 (hr COX-2) purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis MO, U.S.A.). The efficacies of the tested compounds were determined as the concentration causing 50% enzyme inhibition (IC_{50}) (Table 3).

The results indicated that compounds **3b**, **3c** and **4b** were less active (IC_{50} between 87.12 and >100 μmol) than indomethacin ($IC_{50} = 0.26$ μmol) against COX-1. However they showed better inhibitory profiles (IC_{50} between 0.64 and 1.29 μmol) against COX-2 than indomethacin ($IC_{50} = 2.63$ μmol), indicating that they are selective inhibitors for COX-2. To further assess the selectivity profiles of the tested compounds, their approximate selectivity ratios (COX-1/COX-2) were compared to that of the standard COX-2 selective inhibitor, celecoxib. The study showed that compound **3c** was the most selective among the tested compounds with approximate selectivity ratio of 156.25, yet it has lower selectivity compared to that of celecoxib (approximate selectivity ratio of 333).

3.1.2. Ulcerogenic effects

The target compounds were evaluated for their ulcerogenic potential in rats according to Abou Zeit-Har et al. [35]. All the tested compounds proved to have superior GI safety profiles (0–20% ulceration) in the population of test animals at oral dose of 30 μmol/kg per day, as compared to the reference drug (indomethacin), which was found to cause 100% ulceration under the same experimental conditions (Table 1).

Table 3
In Vitro Human COX-2[a] and COX-1[b] enzymes inhibitory activities of compounds **3b**, **3c** and **4c**

Test compound	COX-2 IC_{50}[c] (μmol)	COX-1 IC_{50}[c] (μmol)	Approximate selectivity ratio COX-1/COX-2
Indomethacin	2.63	0.26	0.098
Celecoxib	<0.3	>100	333
3b	1.16	87.12	75.10
3c	0.64	>100	156.25
4b	1.29	96.25	74.60

[a] Human recombinant COX-2 enzyme.
[b] Human COX-1 enzyme from human platelets.
[c] Values are means of at least four experiments.

3.1.3. Acute toxicity

Compounds **3b**, **3c** and **4c** were further evaluated for their oral acute toxicity in male mice using reported methods [36,37]. The results indicated that most of the tested compounds were nontoxic and well tolerated by experimental animals up to 40 mg/kg. Moreover, these compounds were tested for their toxicity through parenteral route [21], the results revealed that all the tested compounds were nontoxic up to 65 mg/kg.

3.1.4. Antimicrobial activity

The target compounds **3**, **4**, **5**, and **6** were evaluated for their antimicrobial activity against *E. coli* representing Gram-negative bacteria, *S. aureus* representing Gram-positive bacteria and *C. albicans* representing fungi. Microdilution susceptibility test in Müller-Hinton broth (Oxoid) and Sabouraud liquid medium (Oxoid) were used for determination of antibacterial and antifungal activities [38]. The minimal inhibitory concentrations (MIC) listed in Table 4, showed that all the tested compounds have weak or no antifungal activity against *C. albicans* except for compounds **6b** and **6c** that showed half the activity of the antifungal drug used (clotrimazole). Regarding the antibacterial activity compounds **3b** and **4a** displayed comparable antibacterial activity against *E. coli* to that displayed by the reference antibacterial drug used (ampicillin). Compounds **3a**, **4b** and **6a–c**, exhibited only half the activity of ampicillin on *E. coli*. All the tested compounds showed weak or no antibacterial activity against the tested Gram-positive microorganism except for compounds **3a**, **3c**, **4a** and **5b** which displayed half the activity of ampicillin.

4. Conclusions

The present study describes the synthesis of four series of novel pyrazolyl benzenesulfonamide derivatives bearing thiazolyl and thiadiazolyl ring systems. The synthesized compounds exhibited anti-inflammatory activity and superior gastrointestinal safety profile with three compounds (**3b**, **3c** and **4c**) had similar or higher anti-inflammatory activity than the reference compound (indomethacin). These compounds

Table 4
Minimal inhibitory concentrations (MIC μg/ml) of test compounds

Test compound	*E. coli*	*S. aureus*	*C. albicans*
3a	50	25	100
3b	25	100	100
3c	100	25	>200
4a	25	25	>200
4b	50	50	100
4c	100	100	50
5a	100	>200	100
5b	100	25	>200
5c	100	100	>200
6a	50	>200	50
6b	50	100	25
6c	50	>200	25
Ampicillin	25	12.5	–
Clotrimazole	–	–	12.5

(**3b**, **3c** and **4c**) were well tolerated and had selective inhibitory activity towards COX-2 enzyme. Two compounds (**3b** and **4a**) demonstrated comparable antibacterial activity to that of ampicillin against *E. coli*, whereas compounds **3a**, **3c** and **4a** displayed about half the activity of ampicillin against *S. aureus*. The synthesized compounds showed weak or no antifungal activity against *C. albicans* except for compounds **6b** and **6c** that showed half the activity of the control antifungal drug used (clotrimazole). Given the dual functional properties of the reported compounds, they represent a promising class of safer pyrazole containing compounds with interesting pharmacological profile.

5. Experimental

5.1. Chemistry

Melting points were determined in open glass capillaries using Thomas capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on 470-Shimadzu infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on Jeol-400 MHz NMR-spectrometer (DMSO-d_6) and chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard. Splitting patterns were designated as follows: s, singlet; d, doublet; m, multiplet. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer and were found within ±0.4% of the theoretical values. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected aluminium sheets (Type 60 GF 254, Merck) and the spots were detected by exposure to UV–lamp at $\lambda = 254$ nm for few seconds.

5.1.1. N,N-Dimethylaminomethylene-4-(3-phenyl-4-substituted thiosemi carbamoylhydrazonomethyl-1H-pyrazol-1-yl)benzenesulfonamide (2a–c)

To a suspension of *N*,*N*-dimethylaminomethylene-4-(4-formyl-3-phenyl-1*H*-pyrazol-1-yl) benzenesulfonamide **1** (2 g, 5.24 mmol) in ethanol (20 ml) was added an equivalent amount of N^4-substituted thiosemicarbazide. The reaction mixture was heated under reflux for 4 h and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol, dried and crystallized from aqueous dimethylformamide (Table 5).

IR spectra for the target compounds **2a–c** (cm^{-1}): 3327–3281, 3152–3120 (NH); 1622–1626, 1595–1594 (C=N); 1339–1337, 1147–1137 (SO_2); 1548–1521, 1367–1311, 1198–1179, 911–909 (NCS amide I, II III and IV bands). ^{1}H NMR for **2a**: δ 2.93, 3.02 (2s, 6H, N(CH_3)$_2$), 7.22–7.76 (m, 10H, phenyl-H), 7.97, 8.07 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.28 (s, 1H, N=C–H), 8.35 (s, 1H, H–C=N), 9.36 (s, 1H, pyrazole-C_5–H), 9.88 (s, 1H, N^4–H, D_2O exchangeable). 11.83 (s, 1H, N^2–H, D_2O exchangeable). ^{1}H NMR for **2b**; δ 2.33 (s, 3H, tolyl-CH_3), 2.92, 3.16 (2s, 6H, N(CH_3)$_2$), 7.20 (d, $J = 8.8$ Hz, 2H, tolyl-$C_{3,5}$–H), 7.42–7.58 (m, 5H, phenyl-H), 7.73 (d, $J = 8.8$ Hz, 2H, tolyl-$C_{2,6}$–H), 7.96, 8.06 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.26 (s, 1H, N=C–H), 8.33 (s, 1H, H–C=N), 9.34 (s, 1H, pyrazole-C_5–H), 9.80 (s, 1H, N^4–H, D_2O exchangeable), 11.75 (s, 1H, N^2–H, D_2O exchangeable). ^{1}H NMR for **2c**: δ 2.93, 3.16 (2s, 6H, N(CH_3)$_2$), 7.44–7.58 (m, 5H, phenyl-H), 7.66, 7.73, 7.96, 8.06 (4d, $J = 8.8$ Hz, 8H, chlorophenyl-$C_{2,3,5,6}$–H and benzenesulfonamide-$C_{3,5\ \text{and}\ 2,6}$–H), 8.26 (s, 1H, N=C–H), 8.34 (s, 1H, H–C=N), 9.32 (s, 1H, pyrazole-C_5–H), 9.89 (s, 1H, N^4–H, D_2O exchangeable), 11.87 (s, 1H, N^2–H, D_2O exchangeable).

Table 5
Physical data of the target compounds **2–6**

Compound no.	R	Yield %	M.p. °C	Mol. formula (Mol. wt)[a]
2a	C_6H_5	82	231–232	$C_{26}H_{25}N_7O_2S_2$ (531.66)
2b	4-$CH_3C_6H_4$	83	235–236	$C_{27}H_{27}N_7O_2S_2$ (545.69)
2c	4-ClC_6H_4	85	214–215	$C_{26}H_{24}ClN_7O_2S_2$ (566.11)
3a	C_6H_5	75	208–210	$C_{28}H_{25}N_7O_3S_2$ (571.69)
3b	4-$CH_3C_6H_4$	74	254–256	$C_{29}H_{27}N_7O_3S_2$ (585.71)
3c	4-ClC_6H_4	77	222–224	$C_{28}H_{24}ClN_7O_3S_2$(606.13)
4a	C_6H_5	71	155–156	$C_{34}H_{28}BrN_7O_2S_2$ (710.68)
4b	4-$CH_3C_6H_4$	70	149–150	$C_{35}H_{30}BrN_7O_2S_2$ (724.71)
4c	4-ClC_6H_4	72	176–178	$C_{34}H_{27}BrClN_7O_2S_2$ (745.13)
5a	C_6H_5	60	219–220	$C_{26}H_{23}N_7O_2S_2$(529.65)
5b	4-$CH_3C_6H_4$	61	249–250	$C_{27}H_{25}N_7O_2S_2$ (543.68)
5c	4-ClC_6H_4	63	262–264	$C_{26}H_{22}ClN_7O_2S_2$ (564.09)
6a	C_6H_5	83	210–212	$C_{30}H_{29}N_7O_4S_2$ (615.74)
6b	4-$CH_3C_6H_4$	82	205–206	$C_{31}H_{31}N_7O_4S_2$ (629.77)
6c	4-ClC_6H_4	86	232–233	$C_{30}H_{28}ClN_7O_4S_2$ (650.19)

[a] Analyzed for C, H, N, S; results are within ±0.4% of the theoretical values for the formulae given.

5.1.2. N,N-Dimethylaminomethylene-4-[3-phenyl-4-(3-substituted-4-oxothiazolidin-2-ylidenehydrazonomethyl)-1H-pyrazol-1-yl]benzenesulfonamides (3a–c)

To a suspension of the selected thiosemicarbazone **2a–c** (1.9 mmol) in dioxane (20 ml), an equivalent amount of ethyl bromoacetate (0.23 g, 0.16 ml, 1.9 mmol) was added. The reaction mixture was heated under reflux for 4 h, concentrated to approximately half its volume and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol, dried and crystallized from benzene/ethanol (1:2) (Table 5).

IR spectra for the target compounds **3a–c** (cm^{-1}): 1730–1727 (C=O); 1626–1618, 1596–1595 (C=N); 1345–1340, 1147–1146 (SO_2). ^{1}H NMR for **3a**: δ 2.93, 3.16 (2s, 6H, N(CH_3)$_2$), 4.11 (s, 2H, thiazole-C_5–H), 7.30–8.00 (m, 14H, phenyl-H and benzenesulfonamide-H), 8.12 (s, 1H, N=C–H), 8.27 (s, 1H, H–C=N), 9.03 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **3b**: δ 2.36 (s, 3H, tolyl-CH_3), 2.93, 3.16 (2s, 6H, N(CH_3)$_2$), 4.09 (s, 2H, thiazole-C_5–H), 7.16–8.00 (m, 13H, phenyl-H, tolyl-H and benzenesulfonamide-H), 8.19 (s, 1H, N=C–H), 8.26 (s, 1H, H–C=N), 9.02 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **3c**: δ 2.92, 3.16 (2s, 6H, N(CH_3)$_2$), 4.09 (s, 2H, thiazole-C_5–H), 7.41–7.53 (m, 5H, phenyl-H), 7.59, 7.83, 7.93, 8.12 (4d, $J = 8.8$ Hz, 8H, chlorophenyl-$C_{2,3,5,6}$–H and benzenesulfonamide-$C_{3,5\ \text{and}\ 2,6}$–H), 8.26 (s, 1H, N=C–H), 8.28 (s, 1H, H–C=N), 9.02 (s, 1H,

pyrazole-C_5–H). ^{13}C NMR for **3b**: δ 21.14 (tolyl-CH_3), 37.92 ($N(CH_3)_2$, 33.12, 163.53, 168.71 (oxothiazolidine-$C_{5,2,4}$, respectively), 105.94, 127.13, 158.31 (pyrazole-$C_{4,5,3}$, respectively), 127.92, 128.96, 129.52, 137.11 (phenyl-$C_{3,2,4,1}$, respectively), 120.34, 129.23, 135.98, 158.15 (tolyl-$C_{2,3,4,1}$, respectively), 120.21, 128.09, 138.11, 145.38 (benzenesulfonamide-$C_{3,2,1,4}$, respectively), 163.72, 164.11 (SO_2N=CH, CH=NN).

5.1.3. N,N-Dimethylaminomethylene-4-[-3-phenyl-4-[4-(4-bromophenyl)-3-(phenyl or substituted phenyl)-2,3-dihydrothiazol-2-ylidenehydrazonomethyl]-1H-pyrazol-1-yl]-benzenesulfonamides (4a–c)

To a suspension of the selected thiosemicarbazone **2a–c** (1.9 mmol) in dioxane (20 ml), an equivalent amount of 4-bromophenacyl bromide (0.52 g, 1.9 mmol) was added. The reaction mixture was heated under reflux for 5 h, concentrated under vacuum and poured onto crushed ice. The separated solid product was filtered, dried and crystallized from benzene/*n*-hexane (Table 5).

IR spectra for the target compounds **4a–c** (cm^{-1}): 1626–1624, 1595–1594 (C=N); 1342–1338, 1148–1147 (SO_2). ^{1}H NMR for **4a**: δ 2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 6.80 (s, 1H, thiazole-C_5–H), 7.08–7.81(m, 16H, phenyl-H, bromophenyl-H and benzenesulfonamide-$C_{3,5}$–H), 7.92 (d, $J = 8.8$ Hz, 2H, benzenesulfonamide-$C_{2,6}$–H), 8.14 (s, 1H, N=C–H), 8.27 (s, 1H, H–C=N), 8.58 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **4b**: δ 2.29 (s, 3H, tolyl-CH_3), 2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 6.78 (s, 1H, thiazole-C_5–H), 7.10–8.14 (m, 17H, phenyl-H, tolyl-H, bromophenyl-H and benzenesulfonamide-H), 8.59 (s, 1H, N=C–H), 8.95 (s, 1H, H–C=N), 9.45 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **4c**: δ 2.93, 3.17 (2s, 6H, $N(CH_3)_2$), 6.73 (s, 1H, thiazole-C_5–H), 7.10–7.68 (m, 15H, phenyl-H, chlorophenyl-H, bromophenyl-H and benzenesulfonamide-$C_{3,5}$–H), 7.93 (d, $J = 8.8$ Hz, 2H, benzenesulfonamide-$C_{2,6}$–H), 8.12 (s, 1H, N=C–H), 8.16 (s, 1H, H–C=N), 8.95 (s, 1H, pyrazole-C_5–H). ^{13}C NMR for **4b**: δ 20.91 (tolyl-CH_3), 37.68 ($N(CH_3)_2$, 83.51, 143.72, 164.24 (thiazole-$C_{5,4,2}$, respectively), 106.20, 127.81, 158.11 (pyrazole-$C_{4,5,3}$, respectively), 128.72, 128.81, 129.21, 136.51 (phenyl-$C_{3,2,4,1}$, respectively), 120.73, 129.11, 133.91, 152.32 (tolyl-$C_{2,3,4,1}$, respectively), 122.81, 128.14, 131.32, 132.11 (bromophenyl-$C_{4,1,2,3}$, respectively),119.11, 128.20, 137.11, 145.22 (benzenesulfonamide-$C_{3,2,1,4}$, respectively), 163.73, 163.74 (SO_2N=CH, CH=NN).

5.1.4. N,N-Dimethylaminomethylene-4-[3-phenyl-4-(5-substituted amino-1,3,4-thiadiazol-2-yl)-1H-pyrazol-1-yl]-benzenesulfonamide (5a–c)

To a boiling, well stirred solution of the selected thiosemicarbazone **2a–c** (3 mmol) in a mixture of dioxane/ethanol (15 ml, 1:2) was added an aqueous solution of 2 M $FeCl_3$ (0.6 ml) dropwise. The reaction mixture was heated at 70–80 °C for 30 min, concentrated to approximately half its volume, cooled and diluted with water. The separated solid product was filtered, dried and crystallized from ethanol (Table 5).

IR spectra for the target compounds **5a–c** (cm^{-1}): 3189–3176 (NH); 1626, 1595 (C=N); 1344–1341, 1149–1147 (SO_2). ^{1}H NMR for **5a**: δ 2.94, 3.17 (2s, 6H, $N(CH_3)_2$), 6.98–7.78 (m, 10H, phenyl-H), 7.95, 8.16 (2d, J = 8.8 Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.28 (s, 1H, N=C–H), 9.24 (s, 1H, pyrazole-C_5–H), 10.41 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR for **5b**: δ 2.26 (s, 3H, tolyl-CH_3), 2.93, 3.17 (2s, 6H, $N(CH_3)_2$), 7.16 (d, $J = 8.1$ Hz, 2H, tolyl-$C_{3,5}$–H), 7.47–7.54 (m, 5H, phenyl-H), 7.76 (d, $J = 8.1$ Hz, 2H, tolyl-$C_{2,6}$–H), 7.94, 8.16 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.28 (s, 1H, N=C–H), 9.23 (s, 1H, pyrazole-C_5–H), 10.31 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR for **5c**: δ 2.93, 3.17 (2s, 6H, $N(CH_3)_2$), 7.48–7.98 (m, 11H, phenyl-H, chlorophenyl-H and benzenesulfonamide-$C_{3,5}$–H), 8.17 (d, $J = 8.8$ Hz, 2H, benzenesulfonamide-$C_{2,6}$–H), 8.27 (s, 1H, N=C–H), 9.44 (s, 1H, pyrazole-C_5–H), 10.0 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR for **5b**: δ 21.12 (tolyl-CH_3), 37.64 ($N(CH_3)_2$, 162.18, 165.82(thiadiazole-$C_{2,5}$, respectively), 106.22, 127.11, 158.38 (pyrazole-$C_{4,5,3}$, respectively), 128.31, 128.67, 128.99, 137.12 (phenyl-$C_{3,,2,4,1}$, respectively), 120.34, 127.35, 131.14, 144.28 (tolyl-$C_{2,3,4,1}$, respectively), 119.32, 127.38, 138.17, 144.78 (benzenesulfonamide-$C_{3,2,1,4}$, respectively), 163.81 (SO_2N=CH).

5.1.5. N,N-Dimethylaminomethylene-4-[3-phenyl-4-[3-acetyl-5-(N-substituted acetamido)-2,3-dihydro-1,3,4-thiadiazol-2-yl]-1H-pyrazol-1-yl]benzenesulfonamides (6a–c)

A mixture of the selected thiosemicarbazone **2a–c** (1.9 mmol) and acetic anhydride (10 ml) was heated under reflux for 2 h. The mixture was left to cool, water (10 ml) was added and the mixture was stirred for 30 min to decompose excess acetic anhydride. The separated solid product was filtered, dried and crystallized from methanol (Table 5).

IR spectra for the target compounds **6a–c** (cm^{-1}): 1691–1672 (C=O); 1629–1625, 1598–1595 (C=N); 1343–1337, 1148–1140 (SO_2). ^{1}H NMR for **6a**: δ 1.87 (s, 6H, two $COCH_3$), 2.93, 3.16 (2s, 6H, $N(CH_3)_2$), 7.11 (s, 1H, thiadiazole-C_2–H), 7.46–7.78 (m, 10H, phenyl-H), 7.90, 8.06 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.26 (s, 1H, N=C–H), 8.43 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **6b**: δ 1.85, 1.89 (2s, 6H, two $COCH_3$), 2.38 (s, 3H, tolyl-CH_3), 2.92, 3.16 (2s, 6H, $N(CH_3)_2$), 7.10 (s, 1H, thiadiazole-C_2–H), 7.32–7.78 (m, 9H, phenyl-H and tolyl-H), 7.90, 8.06 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.26 (s, 1H, N=C–H), 8.41 (s, 1H, pyrazole-C_5–H). ^{1}H NMR for **6c**: δ 2.31, 2.49 (2s, 6H, two $COCH_3$), 2.91, 3.15 (2s, 6H, $N(CH_3)_2$), 7.32–7.79 (m, 10H, thiadiazole-C_2–H, phenyl-H and chlorophenyl-H), 7.87, 8.07 (2d, $J = 8.8$ Hz, 4H, benzenesulfonamide-$C_{3,5}$–H and -$C_{2,6}$–H), 8.24 (s, 1H, N=C–H), 8.52 (s, 1H, pyrazole-C_5–H). ^{13}C NMR for **6c**: δ 17.81, 18.49 (two acetyl-CH_3), 37.46 ($N(CH_3)_2$), 39.31, 158.31 (thiadiazole-$C_{2,5}$, respectively), 107.28, 127.16, 154.28 (pyrazole-$C_{4,5,3}$, respectively), 128.51, 129.38, 130.21, 136.72 (phenyl-$C_{3,,2,4,1}$, respectively), 122.74, 128.13, 130.72, 138.91 (chlorophenyl-$C_{2,3,4,1}$,

respectively), 119.98, 128.38, 138.14, 144.93 (benzenesulfonamide-$C_{3,2,1,4}$, respectively), 163.38 ($SO_2N{=}CH$), 164.78, 172.31 (two C=O).

5.2. Biological screening

5.2.1. Anti-inflammatory testing

5.2.1.1. Cotton pellet-induced granuloma bioassay. Adult male Sprague–Dawley rats (120–140 g) obtained from Medical Research Institute, Alexandria University, were used. The rats were acclimated one week prior to use and allowed unlimited access to standard rat chow and water. Prior to the start of experiment, the animals were randomly divided into groups of six rats each. Cotton pellet (35 ± 1 mg) cut from dental rolls were impregnated with 0.2 ml (containing 0.01 mmol) of a solution of the test compound in chloroform and the solvent was allowed to evaporate. Each cotton pellet was subsequently injected with 0.2 ml of an aqueous solution of antibiotics (1 mg penicillin G and 1.3 mg dihydrostreptomycin/ml). Two pellets were implanted subcutaneously, one in each axilla of the rat, under mild general anaesthesia. One group of animals received the standard reference indomethacin and the antibiotics at the same level. Pellets containing only the antibiotics were similarly implanted in the control rats. Seven days later, the animals were sacrificed and the two cotton pellets, with adhering granulomas, were removed, dried for 48 h at 60 °C and weighed. The increment in dry weight (difference between the initial and final weight) was taken as a measure of granuloma ± SEM. This was calculated for each group and the percentage reduction in dry weight of granuloma from control value was also calculated. The ED_{50} values were determined through dose response curves using doses of 4, 7, 10 and 15 μmol for each compound.

5.2.1.2. Carrageenan-induced rat paw edema. Male albino rats weighing 120–150 g (Medical Research Institute, Alexandria University) were used throughout the work. The rats were kept in an animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups of six rats each. The paw edema was induced by subplantar injection of 50 μl of 2% carrageenan solution in saline (0.9%). Indomethacin and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 10 μmol/kg body weight, 1 h prior to carrageenan injection. Control group was injected with DMSO only. The volume of paw edema (ml) was determined by means of plethysmometer immediately after injection of carrageenan and 4 h later. The increase in paw volume from 0 to 4 h was measured [32]. The percentage protection against inflammation was calculated as follows:

$$\frac{V_c - V_d}{V_c} \times 100$$

where V_c is the increase in paw volume in the absence of test compound (control) and V_d is the increase in paw volume after injection of the test compound. Data were expressed as the mean ± SEM. Significant difference between the control and treated groups was determined using Student's *t*-test and *P* values. The difference in results was considered significant when $P < 0.001$. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also calculated.

5.2.1.3. Human COX-1 and COX-2 enzymatic assay. Human COX-1 and COX-2 activities were determined as described by Wakitani et al. [33]. Human COX-1 (0.3 mg protein/assay) or COX-2 (1 mg protein/assay) was suspended in 0.2 ml of 100 mmol tris–HCl buffer (pH 8) containing hematin (2 mmol) and tryptophan (5 mmol) as cofactors. The reaction mixture was pre-incubated with each test compound individually for 5 min at 24 °C. [^{14}C]-arachidonic acid (100.00 dpm, 30 mmol) was added to the mixture and then incubated for 2 min (for COX-1) or 45 min (for COX-2) at 24 °C. The reaction was stopped by the addition of 400 μl of a solution composed of Et_2O/MeOH/1 M citric acid (30:4:1, v/v/v). After centrifugation of the mixture at 1700×/g for 5 min at 4 °C, 50 μl of the upper phase was applied to a thin layer chromatography plate. Thin layer chromatography was performed at 4 °C with solvent system of Et_2O/MeOH/AcOH (90:2:0.1, v/v/v). Enzyme activity was calculated from the percent conversion of arachidonic acid to PGH2 and its decomposition products, using radiometric photographic system. The concentration of the compound causing 50% inhibition (IC_{50}) was calculated.

5.2.2. Ulcerogenic effects

All target compounds were evaluated for their ulcerogenic potential in rats [35]. Indomethacin was used as reference standard. Male albino rats (100–120 g) were fasted for 12 h prior to administration of the compounds. Water was given ad libitum. The animals were divided into groups of six rats each. Control group received 1% gum acacia orally. Other groups received indomethacin or test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 30 μmol/kg body weight per day. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3× magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of stomach lesions [35]. The percentage ulceration for each group was calculated as follows:

$$\%\ \text{Ulceration} = \frac{\text{Number of animals bearing ulcer in a group}}{\text{Total number of animals in the same group}} \times 100$$

5.2.3. Acute toxicity

The oral acute toxicity of compounds **3b**, **3c** and **4c** was investigated using male mice (20 g each, Medical Research

Institute, Alexandria University) according to previously reported methods [36,37]. The animals were divided into groups of six mice each. The compounds were given orally, suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250, 300 mg/kg. The mortality percentage in each group was recorded after 24 h. Additionally, the test compounds were investigated for their parenteral acute toxicity in groups of six mice each as reported earlier [21]. The compounds, or their vehicle propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg. The percentage survival was followed up to seven days [21].

5.2.4. Antimicrobial activity

The microdilution susceptibility test in Muller-Hinton broth (oxoid) and Sabouraud liquid medium (oxoid) were used for the determination of antibacterial and antifungal activities [38]. Test organisms were *Escherichia coli* (*E. coli*) ATCC 25922 as Gram-negative bacteria, *Staphylococcus aureus* (*S. aureous*) ATCC 19433 as Gram-positive bacteria and *Candida albicans* (*C. albicans*) as yeast-like fungi. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at a concentration of 1600 μg/ml. From this stock solution, serial dilutions of the compounds (800, 400,…, 6.25 μg/ml) were prepared. The microorganism suspensions at concentration of 10^6 CFU/ml (colony forming unit/ml) were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h, with the incubation chamber kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MIC) were determined. Controls with DMSO and uninoculated media were run parallel to the tested compounds under same conditions.

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