Monatsh Chem
DOI 10.1007/s00706-015-1549-x

ORIGINAL PAPER

Synthesis and pharmacological evaluation of new pyrazolyl benzenesulfonamides linked to polysubstituted pyrazoles and thiazolidinones as anti-inflammatory and analgesic agents

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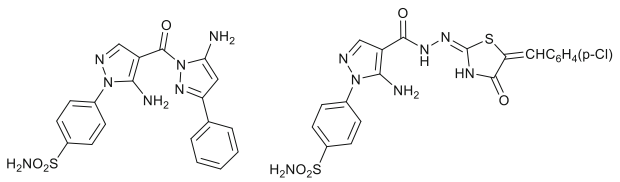
Received: 19 June 2015 / Accepted: 30 July 2015

Abstract New compounds comprising the pyrazolyl benzenesulfonamide scaffold linked to polysubstituted pyrazoles and thiazolidinones were synthesized and evaluated for their anti-inflammatory and analgesic activities. The results revealed that most of the compounds displayed distinctive anti-inflammatory and analgesic activity. Two thiazolidinone derivatives emerged with the highest anti-inflammatory activity in this study, whereas two pyrazole derivatives displayed high analgesic activity with a fast onset of action compared to the reference drug. Moreover, the active compounds showed minimal potential for gastric injury in addition to a good safety margin (ALD_{50} >0.25 g/kg). In vitro COX-1/COX-2 inhibition study revealed that the most active compounds showed relatively more selectivity toward COX-2 than COX-1. Among the test compounds, a thiazolidinone derivative possessed the lowest IC_{50} value against both COX-1 and COX-2.
Graphical abstract



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Keywords Pyrazoles · Thiazolidinones · Anti-inflammatory · Analgesic · COX-1/COX-2 · Bioorganic synthesis

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed agents in the treatment of various inflammatory diseases such as arthritis, rheumatism as well as mild to moderate pain and fever [1]. The pharmacological activity of NSAIDs is closely related to their ability to inhibit both isoforms of the cyclooxygenase enzyme (COX), COX-1 and COX-2 which catalyzes the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs). The constitutive COX-1 isoform is mainly responsible for the synthesis of PGs that exert cytoprotective effect in the gastrointestinal (GI) tract and control renal function in the kidneys, whereas the inducible COX-2 isoform is selectively activated by pro-inflammatory stimuli and facilitates the release of PGs involved in the inflammatory process [2]. Consequently, their long-term clinical use is associated with several side effects such as gastrointestinal lesions, bleeding, and nephrotoxicity [3]. These observations provided an acceptable rationale for the development of selective COX-2 inhibitors that should retain the therapeutic potency of classical NSAIDs with less GI adverse effects [4]. However, recent concerns regarding these drugs and their association with significant cardiovascular side effects led to reconsideration of their appropriate use [5]. Therefore, development of novel compounds having anti-inflammatory activity with an improved safety profile is still a necessity.

Since the discovery of antipyrine, the first pyrazolone derivative used in the management of pain, inflammation, and fever, great attention has been focused on pyrazole derivatives as potent anti-inflammatory, analgesic, and

Published online: 07 September 2015

antipyretic agents [6–10]. As a result, a large number of pyrazoles have been obtained and some have gained application on the clinical level such as celecoxib (Fig. 1), a potent and GI-safe anti-inflammatory and analgesic agent. It is considered as a typical model of the diaryl heterocycle template that is known to selectively inhibit the COX-2 enzyme [11].

On the other hand, the 4-thiazolidinone ring system is one of the privileged structure fragments in modern medicinal chemistry, owing to its broad pharmacological activities and affinity for various biotargets. Among these biological activities, the anti-inflammatory and analgesic activities of thiazolidinones have been of particular interest recently [12–14]. Moreover, some thiazolidinones have been considered as effective lead anti-inflammatory COX-2 inhibitors [15, 16].

In the course of a research program devoted to the development of new anti-inflammatory agents devoid of the undesirable side effects associated with classical NSAIDs, we have reported the synthesis and anti-inflammatory activity of some pyrazolyl benzenesulfonamides substituted with various functionalities and attached to different heterocyclic ring systems through various linkages [17–19]. In particular, potential anti-inflammatory activity and remarkable gastrointestinal safety were displayed by the lead structures **A** [17], **B** [18], and **C** [19] (Fig. 1).

Encouraged by these results, it was attempted in the present study to synthesize and evaluate the anti-inflammatory and analgesic activities of some new structure hybrids comprising basically the pyrazolyl benzenesulfonamide skeleton linked either to polysubstituted pyrazoles at position 4 through a carbonyl bridge (structures **D–G**, Fig. 1), or to substituted thiazolidinones through a carbonyl hydrazinylidene moiety (structure **H**, Fig. 1). The substitution pattern of the pyrazole ring includes various functionalities such as the carbonyl, amino, cyano, and carbethoxy groups in addition to the antipyrine moiety that is documented to be an important integral counterpart in many analgesic and anti-inflammatory agents [6, 20]. Moreover, the incorporated thiazolidinone ring was substituted with a ylidene group which is suggested to be an additive anti-inflammatory pharmacophoric element [12]. In addition to the targeted anti-inflammatory and analgesic activities, the ulcerogenic, acute toxicity profiles and inhibitory activities of COX-1 and COX-2 enzymes were also investigated.

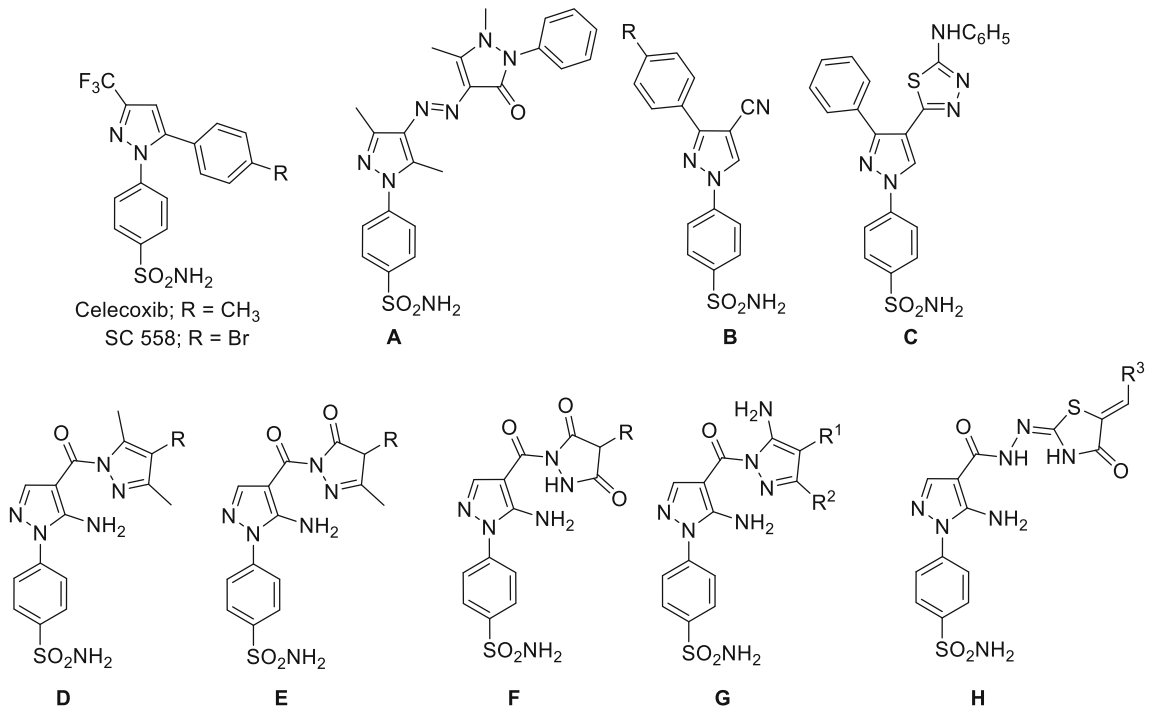


Fig. 1 Chemical structure of reported anti-inflammatory pyrazolyl benzenesulfonamides **A–C** and the newly synthesized compounds **D–H**

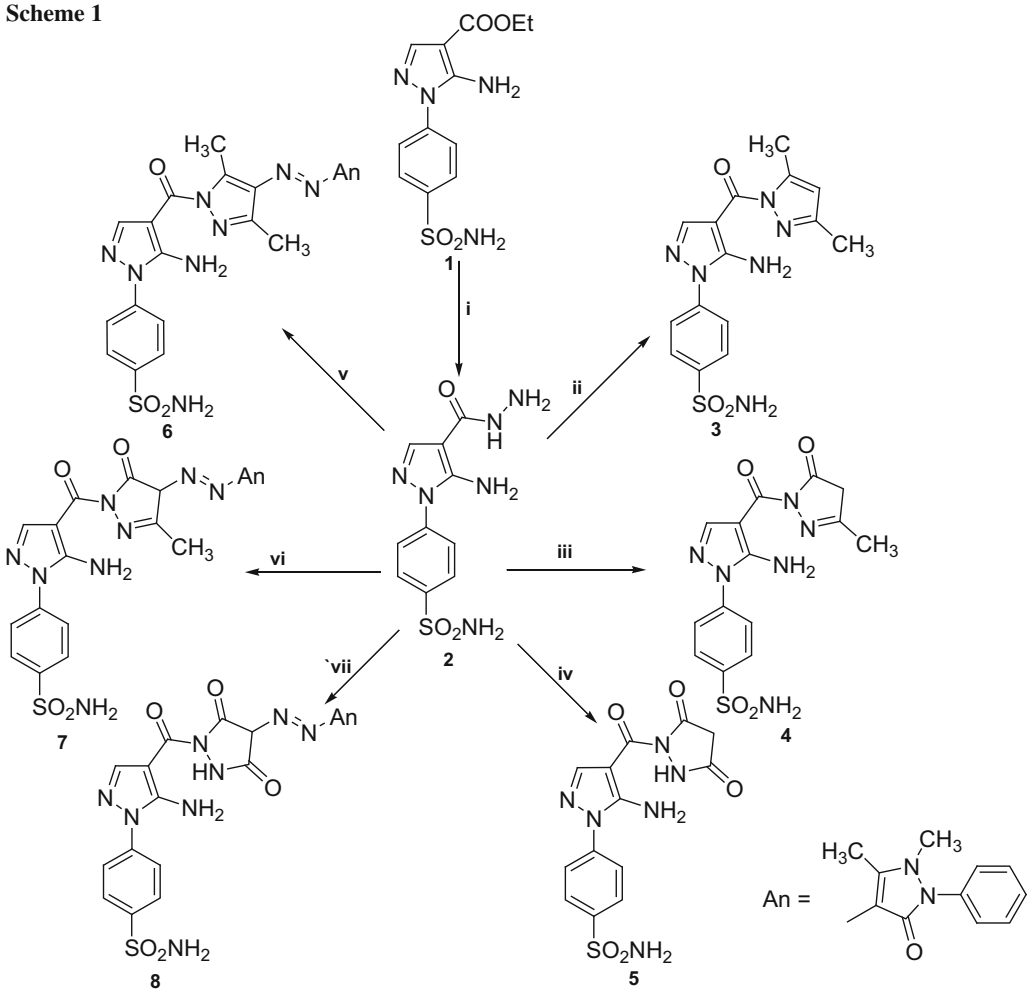
Results and discussion

Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and final compounds are depicted in Schemes 1, 2, and 3. In Scheme 1, the amino ester **1** [21] and the acid hydrazide **2** [22] were prepared as previously described. Condensation of the acid hydrazide **2** with the diketo compounds acetylacetone, ethyl acetoacetate, or diethyl malonate in ethanol/glacial acetic acid mixture yielded the corresponding pyrazole **3** [22], pyrazolinone **4**, and pyrazolidinone **5**, respectively. Similarly, heating **2** with 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)pentane-2,4-dione, ethyl 2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)-3-oxobutanoate, or diethyl 2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)malonate gave rise to the proposed pyrazole **6**, pyrazolinone **7**, and pyrazolidinone **8**, respectively.

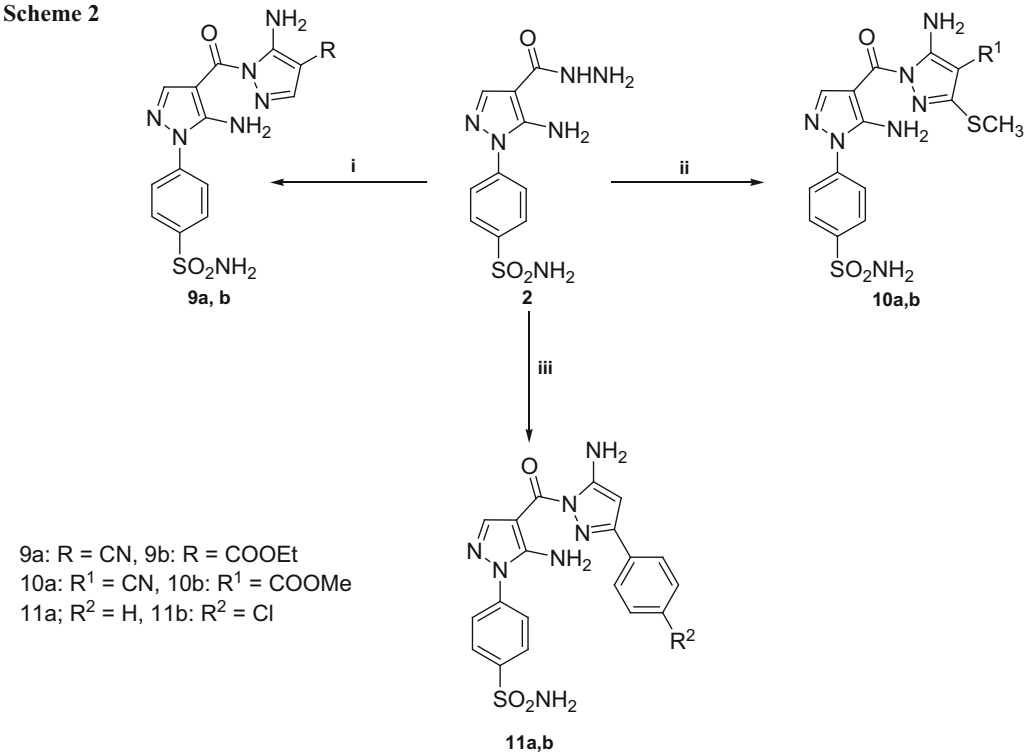
In Scheme 2, heating **2** with ethoxymethylenemalononitrile or ethyl (ethoxymethylene)cyanoacetate in dry DMF resulted in the formation of 5-amino-4-substituted-1*H*-pyrazol-1-yl derivatives **9a**, **9b** in moderate yields. On the other hand, refluxing **2** with [bis(methylthio)methylene]malononitrile or methyl 2-cyano-3,3-bis(methylthio)acrylate in dry DMF following previously published reaction conditions [23] furnished the

Scheme 1



Reagents and Conditions. i)$NH_2NH_2.xH_2O$ 80% / reflux; ii) $CH_3COCH_2COCH_3$ / glacial acetic acid /absolute ethanol / reflux; iii) $CH_3COCH_2COOC_2H_5$ / gl.acetic acid /abs.ethanol/ reflux;iv) $CH_2(COOC_2H_5)_2$ / glacial acetic acid / abs. ethanol/ reflux; v) 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)pentane-2,4-dione / gl. acetic acid / abs. ethanol / reflux; vi) ethyl 2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)-3-oxo butanoate / gl. acetic acid / abs. ethanol/ reflux; vii) diethyl 2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylazo)malonate / gl.acetic acid /abs. ethanol/ reflux

Scheme 2



9a: R = CN, 9b: R = COOEt
10a: R^1 = CN, 10b: R^1 = COOMe
11a; R^2 = H, 11b: R^2 = Cl

Reagents and Conditions: i) $EtOCH=C(CN)_2$ or EtOCH=C(CN)COOEt / DMF / reflux;
ii) $(SCH_3)_2C=C(CN)_2$ or $(SCH_3)_2C=C(CN)COOMe$ / DMF / reflux;
iii) $R^2C_6H_4COCH_2CN$/gl. acetic acid / abs. ethanol / reflux

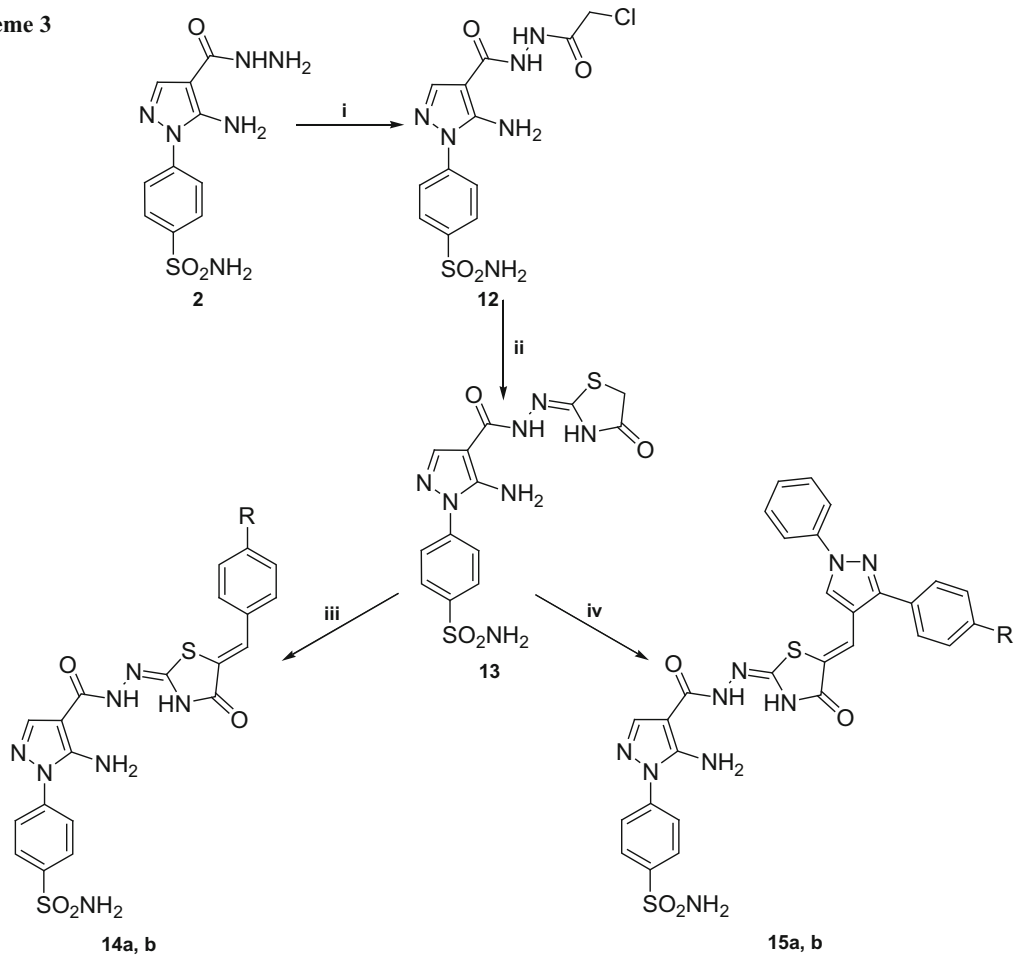
proposed 5-amino-3-methylsulfanyl-4-substituted pyrazolyl derivatives **10a**, **10b**. Synthesis of the target 3-aryl-5-aminopyrazolyl derivatives **11a**, **11b** was achieved by refluxing **2** with phenacyl or 4-chlorophenacyl cyanides in ethanol/glacial acetic acid mixture as described for the synthesis of analogous compounds [22].

In Scheme 3, stirring **2** with chloroacetyl chloride in dry DMF at room temperature furnished the corresponding chloroacetyl derivative **12,** which upon cyclization with ammonium thiocyanate in boiling ethanol produced thiazolidin-4-one **13** in excellent yield and purity. Finally, thiazolidinone **13** was allowed to undergo Knoevenagel condensation with aromatic aldehydes or 3-aryl-1-phenyl-1*H*-pyrazole-4-carboxaldehydes [24] to give the target arylidene derivatives **14** and **15**, respectively. Due to poor solubility of compound **13** in organic solvents, conventional heating in glacial acetic acid in the presence of anhydrous sodium acetate [25] or in ethanol in the presence of piperidine [26] as previously described for synthesis of analogous compounds failed to give the target compounds **14** and **15**. However, carrying out the reaction in a mixture of absolute ethanol/dry DMF (2:1) in the presence of piperidine yielded the target arylidene derivatives **14** and **15** in moderate yields. ^{1}H NMR spectra of compounds **13**–**15** revealed signals for NH proton of the thiazolidinone ring at 11.66–12.54 ppm, accounting for a lactam proton [25]. The *Z* configuration of the exocyclic C=C bond in the 5-arylidene derivatives **14** and **15** was confirmed on the basis of ^{1}H NMR spectral analysis, since the methine proton resonated, as expected, at higher chemical shift values due to the deshielding effect of the adjacent C=O, than it would do in *E* isomers, because of the lower deshielding effect of the S atom [25].

Anti-inflammatory (AI) activity

The AI activity of the synthesized compounds was evaluated utilizing the formalin-induced paw edema bioassay using diclofenac sodium (20 mg/kg) as a reference standard anti-inflammatory agent [27]. The data obtained are presented in Table 1 and expressed as mean ± SE. Statistical differences of control and test groups were carried out using the analysis of variance (ANOVA) followed by 'Student–Newman–Keuls multiple comparison test'. They were performed using computer package of the Statistical Analysis System (SAS, 1987), SAS Incorporation Institute.

Scheme 3



a: R = H, b: R = Cl

Reagents and Conditions: i) $ClCOCH_2Cl$ /dry DMF /r.t.; ii) NH_4SCN /ethanol /reflux;
iii) C_6H_5CHO or 4-ClC_6H_4CHO / DMF/ abs.ethanol /piperidine / reflux;
iv) 3-aryl-1-phenyl-1*H*-pyrazole-4-carboxaldehyde / DMF / abs. ethanol / piperidine/ reflux

The difference in results was considered significant when $p < 0.05$.

Formalin-induced paw edema bioassay

In this acute inflammatory model, each test compound was dosed orally (20 mg/kg body weight) 1 h prior to induction of inflammation by formalin injection. Diclofenac sodium was utilized as a reference drug at a dose of 20 mg/kg, po. The anti-inflammatory activity was then calculated 1–4 h after induction of inflammation and presented in Table 1 as the mean paw volume (cm^3) and the percentage anti-inflammatory activity (AI %).

A comparative study of the anti-inflammatory activity of the test compounds relative to the reference drug at different time intervals indicated the following: after 1 h, 13 compounds showed distinctive pharmacokinetic profiles as revealed from their potent and rapid onset of action which was nearly equivalent to or higher than diclofenac sodium at a dose of 20 mg/kg, po. An outstanding AI % was recorded for the pyrazolyl derivatives **4** (52 %) and **5** (56 %) when compared with diclofenac sodium (36 %), whereas the pyrazolyl derivatives **3**, **8**, **9a**, **9b**, **11b** and the thiazolidinones **13**, **14a**, **14b**, **15a**, **15b** showed anti-inflammatory activity (32–40 %) nearly equivalent to the reference drug. The other compounds showed moderate to weak activity. After 2 h interval, the pyrazole **5** and the thiazolidinones **14a**, **14b**, **15a** displayed anti-inflammatory activity (45–48 %) superior to diclofenac sodium (38 %), whereas the pyrazoles **4**, **7**, **8**, **9a**, and **10a** were nearly effective in inhibiting the paw edema with percentage activity of 35–38 % compared to the reference drug. After

Table 1 Anti-inflammatory activity (AI) of the synthesized compounds in formalin-induced rat paw edema bioassay (acute inflammatory model)

Comp.[a]	Volume of edema/cm^{3}[b]				ED_{50}/mg/kg
	0	1 h	2 h	4 h	
Control	0.33 ± 0.02	0.58 ± 0.02	0.64 ± 0.01	0.83 ± 0.03	
3	0.34 ± 0.02	0.49 ± 0.01[c] (40)	0.56 ± 0.03[c] (29)	0.71 ± 0.01[c] (26)[d]	
4	0.32 ± 0.01	0.44 ± 0.02[c] (52)	0.51 ± 0.01[c] (38)	0.62 ± 0.01[c] (40)	
5	0.35 ± 0.02	0.46 ± 0.01[c] (56)	0.51 ± 0.03[c] (48)	0.64 ± 0.02[c] (42)	
6	0.36 ± 0.02	0.57 ± 0.01[c] (16)	0.58 ± 0.01[c] (29)	0.66 ± 0.02[c] (40)	
7	0.34 ± 0.01	0.55 ± 0.02[c] (16)	0.54 ± 0.02[c] (35)	0.57 ± 0.01[c] (54)	17.12[e]
8	0.35 ± 0.01	0.52 ± 0.02[c] (32)	0.55 ± 0.02[c] (35)	0.57 ± 0.01[c] (56)	16.34
9a	0.34 ± 0.01	0.51 ± 0.01[c] (32)	0.53 ± 0.02[c] (38)	0.60 ± 0.02[c] (48)	
9b	0.35 ± 0.02	0.52 ± 0.01[c] (32)	0.55 ± 0.01[c] (35)	0.62 ± 0.01[c] (46)	
10a	0.33 ± 0.01	0.51 ± 0.01[c] (28)	0.53 ± 0.02[c] (35)	0.61 ± 0.02[c] (44)	
10b	0.35 ± 0.02	0.53 ± 0.01[c] (28)	0.56 ± 0.01[c] (32)	0.65 ± 0.01[c] (40)	
11a	0.33 ± 0.02	0.51 ± 0.01[c] (28)	0.53 ± 0.01[c] (35)	0.58 ± 0.02[c] (50)	
11b	0.33 ± 0.01	0.49 ± 0.01[c] (36)	0.54 ± 0.03[c] (32)	0.61 ± 0.01[c] (44)	
13	0.31 ± 0.03	0.46 ± 0.01[c] (40)	0.52 ± 0.01[c] (32)	0.63 ± 0.03[c] (36)	
14a	0.32 ± 0.01	0.48 ± 0.02[c] (36)	0.49 ± 0.03[c] (45)	0.58 ± 0.01[c] (48)	
14b	0.32 ± 0.01	0.47 ± 0.01[c] (40)	0.49 ± 0.01[c] (45)	0.54 ± 0.03[c] (56)	14.54
15a	0.34 ± 0.01	0.49 ± 0.02[c] (40)	0.50 ± 0.02[c] (48)	0.62 ± 0.01[c] (44)	
15b	0.32 ± 0.02	0.47 ± 0.03[c] (40)	0.49 ± 0.02[c] (45)	0.55 ± 0.01[c] (54)	15.13
Diclofenac sodium	0.34 ± 0.02	0.50 ± 0.01[c] (36)	0.53 ± 0.01[c] (38)	0.55 ± 0.02[c] (58)	13.95

[a] Dose levels, po: test compounds and diclofenac sodium (20 mg/kg b.wt.)

[b] Values are expressed as mean ± SE (number of animals = 6 rats)

[c] Significantly different compared to corresponding control, $p \leq 0.05$

[d] Between parentheses (percentage anti-inflammatory activity, AI %)

[e] ED_{50} is the effective dose calculated after 4 h

4 h, the pyrazoles **7**, **8** and the thiazolidinones **14b**, **15b** showed anti-inflammatory activity (54–56 %) nearly equivalent to diclofenac sodium (58 %), whereas the pyrazole **11a** displayed anti-inflammatory activity (50 %) slightly lower than the reference.

The most active compounds **7**, **8**, **14b**, and **15b** were further tested at 5, 10, 20, 40, and 50 mg/kg body weight to determine their ED_{50} values after 4 h interval. The thiazolidinones **14b** and **15b** were found to be nearly equipotent (ED_{50} = 14.54 and 15.13 mg/kg respectively, Table 1) to diclofenac sodium (ED_{50} = 13.95 mg/kg), whereas compounds **7** and **8** were found to be less potent (ED_{50} = 16.34 and 17.12 mg/kg respectively, Table 1).

A deep insight into the structures of the tested compounds revealed that they represent two different series, namely the pyrazole series (compounds **3**–**11**) and the thiazolidinone series (compounds **13**–**15**). Among the pyrazole derivatives **3**–**8**, it was found that replacing one methyl group (compounds **3** and **6**) by carbonyl functionality (compounds **4** and **7**) led to a marked increase in activity, whereas replacement of the second methyl group by carbonyl (compounds **5** and **8**) did not result in a significant change in the overall activity. Moreover, the presence of antipyrinylazo moiety in compounds **6**–**8** greatly enhanced the anti-inflammatory activity after 4 h compared to the corresponding antipyrinylazo free analogs. Concerning the 5-aminopyrazoles **9**–**11**, it was noticed that introduction of methylsulfanyl moiety (compounds **10a**, **10b**) or removal of the cyano or ester group and introduction of phenyl or 4-chlorophenyl group at position 3 (compounds **11a**, **11b**) did not significantly affect the overall activity in this series of compounds.

On the other hand, within the thiazolidinones **13**–**15**, derivatization of the methylene group to a substituted ylidene moiety (compounds **14** and **15**) led to a noticeable enhancement in the overall activity. Moreover, the ylidene derivatives containing 4-chlorophenyl group (**14b** and **15b**) were more active than their unsubstituted phenyl analogs (**14a** and **15a**) after 4 h intervals.

Collectively, compounds comprising the antipyrine moiety (**7**, **8**) and the thiazolidinones substituted with

Table 2 Analgesic activity of the synthesized compounds using the rat tail withdrawal technique

Comp.[a]	Reaction time/s[b]			
	0	1 h	2 h	4 h
Control	2.86 ± 0.20	2.90 ± 0.10	2.97 ± 0.20	3.01 ± 0.20
3	2.38 ± 0.20	2.82 ± 0.10 (18)[d]	3.06 ± 0.20[c] (32)	3.38 ± 0.20[c] (42)
4	2.75 ± 0.20	3.20 ± 0.10[c] (16)	3.74 ± 0.20[c] (36)	4.15 ± 0.20[c] (50)
5	2.39 ± 0.10	3.15 ± 0.20[c] (31)	3.68 ± 0.20[c] (53)	3.89 ± 0.10[c] (62)
6	2.38 ± 0.20	2.7 ± 0.20 (13)	2.92 ± 0.20[c] (22)	3.18 ± 0.20[c] (33)
7	3.03 ± 0.10	3.53 ± 0.20 (16)	3.88 ± 0.30[c] (28)	4.25 ± 0.20[c] (40)
8	2.58 ± 0.10	3.02 ± 0.20[c] (17)	3.65 ± 0.20[c] (41)	3.85 ± 0.10[c] (49)
9a	2.68 ± 0.20	3.63 ± 0.20[c] (35)	3.88 ± 0.40[c] (44)	4.25 ± 0.20[c] (58)
9b	2.33 ± 0.10	3.00 ± 0.20[c] (28)	3.45 ± 0.20[c] (48)	3.69 ± 0.30[c] (58)
10a	2.65 ± 0. 10	3.32 ± 0.20 (25)	3.65 ± 0.30[c] (38)	3.88 ± 0.20[c] (46)
10b	2.63 ± 0.20	3.23 ± 0.10 (22)	3.54 ± 0.20[c] (34)	3.74 ± 0.20[c] (42)
11a	2.31 ± 0.20	3.10 ± 0.10[c] (34)	3.52 ± 0.20[c] (52)	3.78 ± 0.20[c] (63)
11b	2.66 ± 0.20	3.78 ± 0.20[c] (42)	3.99 ± 0.30[c] (50)	4.25 ± 0.30[c] (59)
13	2.83 ± 0.20	3.05 ± 0.10 (07)	3.32 ± 0.20[c] (17)	3.63 ± 0.20[c] (28)
14a	2.34 ± 0.20	3.04 ± 0.20[c] (30)	3.27 ± 0.20[c] (40)	3.85 ± 0.20[c] (64)
14b	2.58 ± 0.20	3.06 ± 0.30[c] (18)	3.45 ± 0.20[c] (33)	3.70 ± 0.20[c] (43)
15a	2.35 ± 0.20	3.02 ± 0.20[c] (28)	3.25 ± 0.20[c] (38)	3.85 ± 0.20[c] (63)
15b	2.79 ± 0.10	3.08 ± 0.20 (10)	3.19 ± 0.20[c] (14)	3.74 ± 0.20[c] (34)
Diclofenac sodium	2.79 ± 0.20	3.25 ± 0.20[c] (16)	3.99 ± 0.10[c] (43)	4.51 ± 0.30[c] (61)

[a] Dose levels, po: test compounds (20 mg/kg b.wt.), diclofenac sodium (20 mg/kg b.wt.)

[b] Values are expressed as mean ± SE (number of animals = 6 rats)

[c] Significantly different compared to corresponding control, $p \leq 0.05$

[d] Between parentheses (percentage analgesic activity)

chlorobenzylidene group (**14b**, **15b**) displayed anti-inflammatory activity equivalent to diclofenac sodium after 4 h.

Analgesic activity

The analgesic activity of the synthesized compounds was evaluated using the rat tail withdrawal technique in response to immersion in water at 55 °C using diclofenac sodium as a reference drug (20 mg/kg, po) [28]. The analgesic activity was measured at 1–4 h time intervals after pain induction. The results were recorded as the average values of six administrations and the percentage increase of the reaction time in comparison with the basal values. The results are presented in Table 2 and expressed as mean ± SE. Statistical differences of control and test groups were carried out as described in the "Experimental".

A comparative study of the analgesic activity of the test compounds relative to the reference drug at different time intervals revealed the following: after 1 h, the pyrazoles **5**, **9a**, **9b**, **10a**, **10b**, **11a**, **11b** and the thiazolidinones **14a**, **15a** exhibited fast analgesic activity (22–42 %) superior to diclofenac sodium (16 %), whereas the pyrazoles **3**, **4**, **6**, **7**, **8** were nearly equipotent to it. After 2 h, the pyrazoles **5**, **9b**, **11a**, **11b** exhibited analgesic activity (48–53 %) higher than the reference drug (43 %), while the pyrazoles **8**, **9a** and the thiazolidinone **14a** showed similar activity (40–44 %), and the pyrazole **10a** and the thiazolidinone **15a** displayed slightly lower activity (38 %). After 4 h, the pyrazoles **5**, **9a**, **9b**, **11a**, **11b** and the thiazolidinones **14a**, **15a** were found to be nearly equipotent (58–64 %) to the reference drug.

Based on the results in Table 2, the following preliminary structure–activity relationships could be tentatively deduced. Among the pyrazoles **3**–**8**, replacing the two methyl groups by carbonyl functionality (compounds **5** and **8**) markedly improved the anti-inflammatory activity at all time intervals. Unexpectedly, pyrazoles containing the antipyrinylazo moiety (compounds **6**–**8**) were less active than their corresponding antipyrinylazo free analogs (compounds **3**–**5**). Concerning the 5-aminopyrazoles **9**–**11**, it was noticed that introduction of methylsulfanyl moiety (compounds **10a**, **10b**) markedly decreased the activity at all time intervals, whereas removal of the cyano or ester group and introduction of phenyl or 4-chlorophenyl group

at position 3 (compounds **11a**, **11b**) improved the overall activity.

On the other hand, among the thiazolidinones **13–15**, derivatization of the methylene group to a substituted ylidene moiety led to a marked improvement in the overall activity. Moreover, the ylidene derivatives containing unsubstituted phenyl group (**14a** and **15a**) were more active than their 4-chlorophenyl analogs (**14b** and **15b**). Thus, compound **14a** emerged as the most potent derivative among the thiazolidinones **13–15**. Collectively, the pyrazoles **5**, **9a**, **9b**, **11a**, **11b** and the thiazolidinones **14a**, **15a** were the most active analgesic agents in this study with a fast onset of action compared to the reference drug.

Comparing the experimental results from both anti-inflammatory and analgesic screening, it could be concluded that the parameters that modulate one activity were different from those which modulate the other one. This could explain the observation that highly active compounds in anti-inflammatory screening, e.g., compounds **14b** and **15b,** were not potent in the analgesic screening and vice versa.

Ulcerogenic activity

The tested compounds **5**, **7**, **8**, **9a**, **9b**, **11a**, **11b**, **14a**, **14b**, **15a**, and **15b** that exhibited promising anti-inflammatory or analgesic profiles in the pre-mentioned animal models were further evaluated for their ulcerogenic potential in rats [29]. Gross observation of the isolated rat stomachs showed weak ulcerogenic potential of the test compounds at an oral dose of 250 mg/kg, when administered twice at 2 h interval in fasted rats compared with diclofenac sodium which caused serious gastric ulcers under the same experimental conditions.

Acute toxicity

The same selected compounds, **5**, **7**, **8**, **9a**, **9b**, **11a**, **11b**, **14a**, **14b**, **15a**, and **15b,** were further evaluated for their approximate acute lethal dose ALD_{50} in male rats following a reported literature method [30]. The results indicated the tested compounds were non-toxic and showed a high safety margin when screened at graded doses (0.1–0.25 g/kg, po), where their ALD_{50} values were found to be >0.25 g/kg.

In vitro COX-1/COX-2 inhibition assay

The compounds which showed significant in vivo activity were further evaluated for their ability to inhibit COX-1 and COX-2 isoenzymes by in vitro colorimetric COX (ovine) inhibitor assay method [31], which utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD), which is produced during the reduction of PGG_2 to PGH_2, at 590 nm. The efficacies of the tested compounds were determined as the concentration causing 50 % enzyme inhibition (IC_{50}) and recorded in Table 3. The data revealed that the pyrazoles **5**, **7**, **8**, **9a**, **9b**, **11a**, **11b** and the thiazolidinones **14a**, **14b**, **15a**, and **15b** showed relatively more selectivity toward COX-2 than COX-1. Among the tested compounds, the thiazolidinone derivative **14b** demonstrated the highest activity as it showed IC_{50} value of 4.5 μM for COX-1 and 1.06 μM for COX-2.

Table 3 In vitro COX-2 selectivity of the synthesized compounds

Comp	IC_{50}/μM[a]		COX-1/COX-2[b]
	COX-1	COX-2	
5	11.59	2.24	5.17
7	16.0	5.35	2.99
8	25.86	8.23	3.14
9a	12.32	3.60	3.42
9b	26.66	6.90	3.86
11a	22.5	6.33	3.55
11b	20.3	7.30	2.78
14a	5.6	1.52	3.68
14b	4.5	1.06	4.24
15a	18.2	5.0	3.64
15b	28.5	8.98	3.17
Diclofenac sod	0.06	0.22	0.27
Celecoxib	28.5	0.07	407

[a] Values are means of three determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 760111, Cayman Chemicals, MI, USA) and the deviation from the mean is <10 % of the mean value

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50})

Conclusion

The present study reports the synthesis and investigation of the anti-inflammatory and analgesic activities of new pyrazolyl benzenesulfonamides linked to polysubstituted pyrazole and thiazolidinone ring systems. The obtained results revealed that the thiazolidinones **14b** and **15b** were the most potent anti-inflammatory agents in this study, whereas the pyrazoles **11a** and **11b** were the most active analgesic agents with a fast onset of action compared to the reference drug. Additionally, the active compounds revealed lower ulcerogenic potential than the reference drug diclofenac sodium and were well tolerated by experimental animals with a high safety margin (ALD_{50}

>0.25 g/kg). In vitro COX-1/COX-2 inhibition study revealed that the most active compounds showed relatively more selectivity toward COX-2 than COX-1 which might account for their low ulcerogenic potential. Among the test compounds, compound **14b** demonstrated the highest activity with IC_{50} value of 4.5 μM for COX-1 and 1.06 μM for COX-2.

Finally, these compounds represent a new structure scaffold that could be further optimized for future development of more potent anti-inflammatory and/or analgesic agents with better GIT tolerance.

Experimental

All reagents and solvents were purchased from commercial suppliers and dried and purified when necessary by standard techniques. Melting points were determined in open glass capillaries using Stuart capillary melting point apparatus (Stuart scientific Stone, Staffordshire, UK). Infrared (IR) spectra were recorded on Perkin Elmer 1430 infrared spectrophotometer (Perkin Elmer, Beaconsfield, UK) and measured using KBr cell. NMR spectra were scanned on Varian Mercury VX-300 or VX-400 using tetramethylsilane (TMS) as internal standard and DMSO-d_6 as solvent (chemical shifts are given in δ/ppm). Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV) or on a gas chromatograph/mass spectrometer Schimadzu GCMS-QP 2010 Plus (70 eV). Microanalyses were performed at the regional Center for Mycology and Biotechnology, El-azhar University, and the found values were within ±0.3 % of the theoretical values. Follow-up of the reactions and checking the purity of the compounds were made by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60 GF254, Merck, Germany) and the spots were detected by exposure to UV lamp at $\lambda = 254$ nm for a few seconds.

*Ethyl 5-amino-1-(4-sulfamoylphenyl)-1H-pyrazole-4-carboxylate (**2**)*

A mixture of 16.92 g ethyl ethoxymethylenecyanoacetate (100 mmol), 22.37 g sulfanilamide hydrazine hydrochloride (100 mmol), and 8.2 g anhydrous sodium acetate (100 mmol) in 50 cm^3 ethanol was heated under reflux for 3 h. The reaction mixture was left overnight and the separated solid product was filtered, washed with ethanol, dried, and recrystallized from ethanol. White crystals (70 %); m.p.: 231–232 °C (Ref. [21] 230 °C).

*4-[5-Amino-4-(hydrazinocarbonyl)-1H-pyrazol-1-yl]benzenesulfonamide (**3**)*

A suspension of 6.2 g **2** (20 mmol) in 30 cm^3 hydrazine hydrate (80 %) was heated under reflux for 8 h. The reaction mixture was left overnight and the separated solid product was filtered, washed with ethanol, dried, and recrystallized from dimethyl formamide/ethanol as white crystals (82 %). M.p.: 297 °C (Ref. [22] 296–297 °C).

General procedure for the synthesis of 4-[5-amino-4-substituted-1*H*-pyrazol-1-yl]benzenesulfonamides 3–8

A mixture of 0.59 g acid hydrazide **2** (2 mmol) and the selected diketo derivative (2 mmol) in 20 cm^3 of glacial acetic acid/absolute ethanol mixture (2:1) was heated under reflux for 8–12 h. The reaction mixture was allowed to attain room temperature and the separated solid product was filtered, dried, and crystallized from the proper solvent.

*4-[5-Amino-4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**3**)*

White crystals (0.6 g, 84 %); m.p.: 244 °C (Ref. [22] 244–245 °C).

4-[5-Amino-4-[(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**4**, $C_{14}H_{14}N_6O_4S$)

Grayish white crystals (0.46 g, 64 %); m.p: >300 °C (dimethyl formamide/ethanol 4:1); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.16$ (s, 3H, CH_3), 4.0 (s, 2H, CH_2, keto tautomer), 6.62 (s, 2H, pyrazole NH_2, D_2O exchangeable), 7.49 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.80, 7.96 (2d, $J = 8.3$ Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.09 (s, 1H, pyrazole C_3–H), 9.82 (s, 1H, OH, enol tautomer) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 14.93$ (CH_3), 59.60 (CH_2), 95.59 (pyrazole C_4), 124.24, 127.49, 140.90, 141.43 (benzenesulfonamide-C), 142.43, 142.98 (pyrazole and pyrazolinone C_3), 150.54 (pyrazole C_5), 162.82, 164.69 (C=O) ppm; IR (KBr): $\bar{\nu} = 3406$, 3325, 3188 (OH, NH), 3071, 2985 (CH), 1670, 1650 (C=O), 1619, 1589 (C=N), 1544, 1495, 1438 (C=C), 1326, 1163 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 362 (M^+, 78.0), 125 (100.0).

4-[5-Amino-4-[(3,5-dioxopyrazolidin-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**5**, $C_{13}H_{12}N_6O_5S$)

Grayish white crystals (0.5 g, 69 %); m.p: 259–260 °C (dimethyl formamide/ethanol 2:1); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.28$ (s, 2H, CH_2), 6.51 (s, 2H, pyrazole NH_2, D_2O exchangeable), 7.45 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.78 (d, $J = 7.7$ Hz, 2H, benzenesulfonamide $C_{3,5}$–H), 7.92–7.95 (m, 3H, benzenesulfonamide $C_{2,6}$–H and pyrazole C_3–H), 9.17 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 20.65$ (CH_2), 95.88 (pyrazole C_4), 123.23, 127.11, 139.14, 140.69 (benzenesulfonamide-C), 142.29 (pyrazole

C_3), 150.12 (pyrazole C_5), 163.50, 169.06, 174.90 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3387, 3343, 3221 (NH), 3018 (CH), 1692 (C=O), 1623 (C=N), 1543, 1507, 1434 (C=C), 1327, 1164 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 364 (M^+, 76.0), 247 (100.0).

4-[5-Amino-4-[[4-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)azo]-3,5-dimethyl-1H-pyrazol-1-yl]-carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide
(**6**, $C_{26}H_{26}N_{10}O_4S$)
Orange crystals (0.85, 74 %); m.p: 276–277 °C (dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.48, 2.62 (2 s, each 3H, pyrazole CH_3), 2.80 (s, 3H, pyrazolinone C–CH_3), 3.35 (s, 3H, pyrazolinone N–CH_3), 6.92 (s, 2H, pyrazole NH_2, D_2O exchangeable), 7.18–7.59 (m, 7H, phenyl-H and SO_2NH_2), 7.80, 7.97 (2d, J = 8.0 Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.53 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 11.25, 12.50, 31.25 (C–CH_3), 36.25 (N–CH_3), 96.32, 96.36, 109.70 (dimethylpyrazole C_4, aminopyrazole C_4, and dimethylpyrazolinone C_4), 123.64, 123.78, 124.33, 127.48, 127.56, 129.71, 140.0, 140.94 (benzenesulfonamide-C and phenyl-C), 142.82, 142.86 (aminopyrazole C_3 and dimethylpyrazole C_3), 150.0, 152.50, 153.75 (aminopyrazole C_5, dimethylpyrazole C_5, and dimethylpyrazolinone C_5), 162.70, 162.79 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3325, 3289, 3175 (NH), 3073, 2975, 2931 (CH), 1669, 1641 (C=O), 1591 (C=N), 1537, 1493, 1428 (C=C), 1352, 1158 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 574 (M^+, 76.0), 247 (100.0).

4-[5-Amino-4-[[4-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)azo]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**7**, $C_{25}H_{24}N_{10}O_5S$)
Reddish crystals (0.83 g, 72.3 %); m.p.: 266–267 °C (dimethyl formamide/ethanol 4:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.26 (s, 3H, methylpyrazolinone CH_3), 2.44 (s, 1H, methylpyrazolinone C_4–H), 2.54 (s, 3H, dimethylpyrazolinone C–CH_3), 3.18 (s, 3H, dimethylpyrazolinone N–CH_3), 7.15 (s, 2H, pyrazole NH_2, D_2O exchangeable), 7.36–7.57 (m, 7H, phenyl-H and SO_2NH_2), 7.79, 7.97 (2d, J = 8.4 Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.30 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 12.14, 31.33 (C–CH_3), 36.46 (N–CH_3), 83.28 (methylpyrazolinone C_4), 96.30, 109.63 (aminopyrazole C_4 and dimethylpyrazolinone C_4), 123.62, 123.82, 124.29, 127.64, 127.92, 129.78, 140.22, 140.89 (benzenesulfonamide-C and phenyl-C), 142.76, 142.88 (aminopyrazole C_3 and methylpyrazolinone C_3), 150.65, 153.84 (aminopyrazole C_5 and dimethylpyrazolinone C_5), 162.50, 162.70, 162.79 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3337, 3325 (NH), 3075, 2975, 2900 (CH), 1695, 1631 (C=O), 1613, 1588 (C=N), 1546, 1525, 1498 (C=C), 1323, 1165 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 576 (M^+, 84.0), 125 (100.0).

4-[5-Amino-4-[[4-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)azo]-3,5-dioxopyrazolidin-1-yl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide
(**8**, $C_{24}H_{22}N_{10}O_6S$)
Orange crystals (0.71 g, 62 %); m.p.: 236–238 °C (dimethyl formamide/ethanol 2:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s, 1H, pyrazolidinone C_4–H), 2.58 (s, 3H, dimethylpyrazolinone C–CH_3), 3.19 (s, 3H, dimethylpyrazolinone N–CH_3), 7.15 (s, 2H, pyrazole NH_2, D_2O exchangeable), 7.39–7.58 (m, 7H, phenyl-H and SO_2NH_2), 7.79, 7.99 (2d, J = 8.4 Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.32 (s, 1H, pyrazole C_3–H), 9.17 (s, 1H, NH, D_2O exchangeable) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 31.33 (C–CH_3), 36.46 (N–CH_3), 84.24, 96.27, 109.46 (pyrazolidinone C_4, aminopyrazole C_4, and dimethylpyrazolinone C_4), 123.31, 123.94, 124.38, 127.45, 127.98, 129.72, 140.47, 141.13 (benzenesulfonamide-C and phenyl-C), 142.57 (aminopyrazole C_3), 151.35, 153.16 (aminopyrazole C_5 and dimethylpyrazolinone C_5), 162.84, 163.16, 164.45, 165.50 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3376, 3333, 3246 (NH), 3016 (CH), 1692, 1646 (C=O), 1623 (C=N), 1547, 1516, 1435 (C=C), 1326, 1161 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 578 (M^+, 73.0), 125 (100.0).

General procedure for the synthesis of 4-[5-amino-4-[(5-amino-4-substituted-1*H*-pyrazol-1-yl)carbonyl]-1*H*-pyrazol-1-yl]benzenesulfonamides 9a, 9b

A mixture of 0.59 g **2** (2 mmol) and ethoxymethylene malononitrile or ethyl ethoxymethylenecyanoacetate (2 mmol) in 10 cm^3 dry DMF was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure and poured into ice-cold water. The obtained precipitate was filtered, washed with ethanol, dried, and crystallized from the proper solvent.

4-[5-Amino-4-[(5-amino-4-cyano-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide
(**9a**, $C_{14}H_{12}N_8O_3S$)
Grayish white crystals (0.5 g, 68 %); m.p.: >300 °C (dimethyl formamide/water 2:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.89 (s, 2H, NH_2, D_2O exchangeable), 6.70 (s, 2H, NH_2, D_2O exchangeable), 7.49 (s, 2H, SO_2NH_2, D_2O exchangeable), 8.0, 8.31 (2d, J = 9.2 Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.48, 8.58 (2 s, each 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 93.81, 94.12 (pyrazole C_4), 112.30 (CN), 124.82, 127.95, 140.66, 143.13 (benzenesulfonamide-C), 143.74, 142.98 (pyrazole C_3), 153.11, 154.36 (pyrazole C_5), 163.33 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3337,

3255 (NH), 3045 (CH), 2202 (CN), 1697 (C=O), 1632 (C=N), 1597, 1574, 1528, 1432 (C=C), 1335, 1163 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 372 (M^+, 100.0).

Ethyl 5-amino-1-[[5-amino-1-(4-sulfamoylphenyl)-1H-pyrazol-4-yl]carbonyl]-1H-pyrazole -4-carboxylate (**9b**, $C_{16}H_{17}N_7O_5S$)

Beige crystals (0.53 g, 64 %); m.p.: 258–259 °C (dimethyl formamide/ethanol 2:1); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.27$ (t, $J = 6.9$ Hz, 3H, CH_3), 4.23 (q, $J = 6.9$ Hz, 2H, CH_2), 7.29, 7.45 (2 s, each 2H, 2 NH_2, D_2O exchangeable), 7.54 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.79 (d, $J = 8.9$ Hz, 2H, benzenesulfonamide $C_{2,6}$–H), 7.85 (s, 1H, pyrazole C_3–H), 7.98 (d, $J = 8.9$ Hz, 2H, benzenesulfonamide $C_{3,5}$–H), 8.50 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 14.89$ (CH_3), 59.72 (CH_2), 93.81, 96.17 (pyrazole C_4), 124.43, 127.53, 140.32, 143.15 (benzenesulfonamide-C), 143.40, 143.68 (pyrazole C_3), 153.37, 154.05 (pyrazole C_5), 163.45, 164.45 (C=O) ppm; IR (KBr): $\bar{\nu} = 3378$, 3334 (NH), 3056 (CH), 1688, 1667 (C=O), 1625 (C=N), 1527, 1486 (C=C), 1331, 1161 (SO_2) cm^{-1}; MS (70 eV): *m/z* (%) = 374 (M^+–C_2H_5O, 1.3), 266 (100 %).

General procedure for the synthesis of 4-[5-amino-4-[(5-amino-3-methylsulfanyl-4-substituted-1*H*-pyrazol-1-yl)carbonyl]-1*H*-pyrazol-1-yl]benzenesulfonamides 10a, 10b

A mixture of 0.59 g **2** (2 mmol) and [bis(methylthio)methylene]malononitrile or methyl 2-cyano-3,3-bis(methylthio)acrylate (2 mmol) in 10 cm^3 dry DMF was heated under reflux for 4 h. The reaction mixture was evaporated under reduced pressure and the remaining residue was triturated with ethanol. The separated solid product was filtered, washed with ethanol, dried, and crystallized from dimethyl formamide/ethanol (3:1).

4-[5-Amino-4-[(5-amino-4-cyano-3-methylsulfanyl-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**10a**, $C_{15}H_{14}N_8O_3S_2$)

White crystals (0.58 g, 70 %); m.p.: 283–284 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.61$ (s, 3H, SCH_3), 7.23 (s, 2H, NH_2, D_2O exchangeable), 7.50 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.78, 7.96 (2d, $J = 8.7$ Hz, each 2H, benzenesulfonamide $C_{3,5}$ and $C_{2,6}$–H), 8.07 (s, 2H, NH_2, D_2O exchangeable), 8.46 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 13.20$ (CH_3), 72.44, 95.99 (pyrazole C_4), 113.50 (CN), 124.41, 127.55, 140.35, 143.38 (benzenesulfonamide-C), 143.47, 153.13 (pyrazole C_3), 153.39, 156.65 (pyrazole C_5), 163.36 (C=O) ppm; IR (KBr): $\bar{\nu} = 3439$, 3311 (NH), 2922 (CH), 2203 (CN), 1659 (C=O), 1615 (C=N), 1529, 1502, 1467 (C=C), 1362, 1154 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 418 (M^+, 30.5), 416 (100.0).

Methyl 5-amino-1-[[5-amino-1-(4-sulfamoylphenyl)-1H-pyrazol-4-yl]carbonyl]-3-methylsulfanyl-1H-pyrazole-4-carboxylate (**10b**, $C_{16}H_{17}N_7O_5S_2$)

White crystals (0.63 g, 70 %); m.p.: 291–292 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.49$, 3.74 (2 s, each 3H, SCH_3 and OCH_3), 7.25 (s, 2H, NH_2, D_2O exchangeable), 7.52 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.57 (s, 2H, NH_2, D_2O exchangeable), 7.79, 7.98 (2d, $J = 8.7$ Hz, each 2H, benzenesulfonamide $C_{2,6}$ and $C_{3,5}$–H), 8.52 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 13.11$ (SCH_3), 51.24 (OCH_3), 91.83, 96.20 (pyrazole C_4), 124.32, 127.81, 140.43, 143.29 (benzenesulfonamide-C), 143.50, 153.29 (pyrazole C_3), 153.35, 155.16 (pyrazole C_5), 163.51, 163.77 (C=O) ppm; IR (KBr): $\bar{\nu} = 3411$, 3343, 3297, 3208 (NH), 3096, 2960 (CH), 1679 (C=O), 1617 (C=N), 1515, 1436, 1400 (C=C), 1335, 1158 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 451 (M^+–CH_3O, 0.88), 265 (100).

General procedure for the synthesis of 4-[5-amino-4-[(5-amino-3-aryl-1*H*-pyrazol-1-yl)carbonyl]-1*H*-pyrazol-1-yl]benzenesulfonamides 11a, 11b

To a suspension of 0.59 g **2** (2 mmol) in 20 cm^3 ethanol/acetic acid mixture (4:1), the selected phenacyl cyanide (2 mmol) was added. The reaction mixture was heated under reflux for 12 h during which a crystalline precipitate separated out. The reaction mixture was cooled, filtered, washed with ethanol, and recrystallized from dimethyl formamide/ethanol (2:1).

4-[5-Amino-4-[(5-amino-3-phenyl-1H-pyrazol-1-yl)carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**11a**, $C_{19}H_{17}N_7O_3S$)

White fine crystals (0.55 g, 65 %); m.p.: 258–259 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.87$ (s, 1H, pyrazole C_4–H), 6.78, 7.21 (2 s, each 2H, 2 NH_2, D_2O exchangeable), 7.38–7.47 (m, 3H, phenyl $C_{3,4,5}$–H), 7.50 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.82 (d, $J = 8.4$ Hz, 2H, benzenesulfonamide $C_{3,5}$–H), 7.87 (d, $J = 7.8$ Hz, 2H, phenyl $C_{2,6}$–H), 8.0 (d, $J = 8.4$ Hz, 2H, benzenesulfonamide $C_{2,6}$–H), 8.74 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 84.23$, 94.10 (pyrazole C_4), 123.83, 127.06, 127.58, 128.84, 129.35, 133.36, 140.09, 142.76 (benzenesulfonamide-C and phenyl-C), 143.89, 152.12 (pyrazole C_3), 152.76, 152.98 (pyrazole C_5), 164.19 (C=O) ppm; IR (KBr): $\bar{\nu} = 3461$, 3440, 3335, 3224 (NH), 3060 (CH), 1649 (C=O), 1614 (C=N), 1531, 1494, 1436 (C=C), 1323, 1159 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 423 (M^+, 65.5), 366 (100).

4-[5-Amino-4-[[5-amino-3-(4-chlorophenyl)-1H-pyrazol-1-yl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**11b**, $C_{19}H_{16}ClN_7O_3S$)

Beige crystals (0.55 g, 60 %); m.p.: 263–264 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.88$ (s, 1H, pyrazole C_4–H), 6.84, 7.24 (2 s, each 2H, 2 NH_2, D_2O exchangeable), 7.51 (d, $J = 7.7$ Hz, 2H, chlorophenyl $C_{3,5}$–H), 7.54 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.83 (d, $J = 7.7$ Hz, 2H, benzenesulfonamide $C_{3,5}$–H), 7.90 (d, $J = 7.7$ Hz, 2H, chlorophenyl $C_{2,6}$–H), 8.01 (d, $J = 7.7$ Hz, 2H, benzenesulfonamide $C_{2,6}$–H), 8.74 (s, 1H, pyrazole C_3–H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 84.59$, 96.13 (pyrazole C_4), 123.83, 126.97, 127.09, 127.60, 128.77, 131.25, 140.09, 142.76 (benzenesulfonamide-C and chlorophenyl-C), 143.81, 152.12 (pyrazole C_3), 152.78, 152.93 (pyrazole C_5), 164.19 (C=O) ppm; IR (KBr): $\bar{\nu} = 3386$, 3357, 3308, 3273 (NH), 3062 (CH), 1651 (C=O), 1617 (C=N), 1574, 1529, 1495 (C=C), 1332, 1162 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 459 ($[M + 2]^+$, 27.6), 457 (M^+, 70.8), 425 (100.0).

4-[5-Amino-4-[[2-(2-chloroacetyl)hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**12**, $C_{12}H_{13}ClN_6O_4S$)

To a stirred ice-cooled solution of 2.96 g **2** (10 mmol) in 10 cm^3 dry DMF, 1.25 g chloroacetyl chloride (0.88 cm^3, 11 mmol) was added dropwise. The reaction mixture was left stirred at room temperature overnight and then poured onto crushed ice. The formed precipitate was filtered, dried, and crystallized from ethanol. White crystals (2.9 g, 78 %); m.p.: 144–145 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.18$ (s, 2H, CH_2), 6.62 (s, 2H, NH_2, D_2O exchangeable), 7.49 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.78, 7.95 (2d, $J = 8.9$ Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.03 (s, 1H, pyrazole C_3–H), 9.97, 10.20 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 45.7$ (CH_2), 94.62 (pyrazole C_4), 123.78, 127.63, 140.94, 141.62 (benzenesulfonamide-C), 142.55 (pyrazole C_3), 150.75 (pyrazole C_5), 162.80, 163.45 (C=O) ppm; IR (KBr): $\bar{\nu} = 3358$, 3209 (NH), 3020 (CH), 1692 (C=O), 1623 (C=N), 1575, 1535, 1500, 1431 (C=C), 1328, 1163 (SO_2) cm^{-1}.

4-[5-Amino-4-[[(2Z)-2-(4-oxo-1,3-thiazolidin-2-ylidene)hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**13**, $C_{13}H_{13}N_7O_4S_2$)

A mixture of 3.73 g **12** (10 mmol) and 1.52 g ammonium thiocyanate (20 mmol) in 20 cm^3 ethanol (95 %) was refluxed for 2 h. The reaction mixture was concentrated and diluted with ice-cold water. The obtained precipitate was filtered, dried, and crystallized from dimethyl formamide/water (3:1). White crystals (3.36 g, 85 %); m.p.: 251–252 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.94$ (s, 2H, CH_2), 6.63 (s, 2H, NH_2, D_2O exchangeable), 7.46 (s, 2H, SO_2NH_2, D_2O exchangeable), 7.78, 7.95 (2d, $J = 7.8$ Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.12 (s, 1H, pyrazole C_3–H), 10.26, 11.66 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 44.42$ (thiazolidinone C_5), 105.32 (pyrazole C_4), 132.50, 132.75, 136.43, 148.50 (benzenesulfonamide-C), 149.96 (pyrazole C_3), 151.65 (pyrazole C_5), 159.58 (thiazolidinone C_2), 166.60, 171.77 (C=O) ppm; IR (KBr): $\bar{\nu} = 3453$, 3356, 3275, 3161 (NH), 3009 (CH), 1702 (C=O), 1626 (C=N), 1538, 1474, 1429 (C=C), 1327, 1160 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 395 (M^+, 70.3), 266 (100.0).

General procedure for the synthesis of 4-[5-amino-4-[[(2*E*)-2-[(5*Z*)-5-substituted-4-oxo-1,3-thiazolidin-2-ylidene]hydrazinyl]carbonyl]-1*H*-pyrazol-1-yl]benzenesulfonamides 14 and 15

A mixture of 0.79 g thiazolidinone **13** (2 mmol), the selected aryl aldehyde (2.2 mmol), and piperidine (2 mmol) in 10 cm^3 of dry DMF/absolute EtOH mixture (4:1) was heated under reflux for 12 h. The reaction mixture was allowed to attain room temperature and the separated product was filtered, washed with ethanol, dried, and crystallized from dimethyl formamide/ethanol (2:1).

4-[5-Amino-4-[[(2E)-2-((5Z)-5-benzylidene-4-oxo-1,3-thiazolidin-2-ylidene)hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**14a**, $C_{20}H_{17}N_7O_4S_2$)

Yellow solid (0.5 g, 52 %); m.p.: 291–292 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 6.68$ (s, 2H, NH_2, D_2O exchangeable), 7.42–7.56 (m, 5H, phenyl $C_{3,4,5}$–H and SO_2NH_2), 7.61 (d, $J = 7.5$ Hz, 2H, phenyl $C_{2,6}$–H), 7.65 (s, 1H, = CH), 7.80, 7.96 (2d, $J = 8.7$ Hz, each 2H, benzenesulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.09 (s, 1H, pyrazole C_3–H), 10.58, 12.31 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 104.45$ (pyrazole C_4), 119.54 (thiazolidinone C_5), 126.23, 127.40, 129.13, 131.65, 132.43, 136.38, 137.16, 148.29 (phenyl-C and benzenesulfonamide-C), 149.22 (pyrazole C_3), 149.43 (=CH), 151.27 (pyrazole C_5), 158.14 (thiazolidinone C_2), 166.45, 171.26 (C=O) ppm; IR (KBr): $\bar{\nu} = 3410$, 3328, 3248 (NH), 3010, 2969 (CH), 1686 (C=O), 1645 (C=N), 1599, 1540 (C=C), 1332, 1158 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 483 (M^+, 94.2), 413 (100.0).

4-[5-Amino-4-[[(2E)-2-[(5Z)-5-(4-chlorobenzylidene)-4-oxo-1,3-thiazolidin-2-ylidene]hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**14b**, $C_{20}H_{16}ClN_7O_4S_2$)

Yellow solid (0.5 g, 50 %); m.p: 298–299 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 6.69$ (s, 2H, NH_2, D_2O exchangeable), 7.48–7.57 (m, 6H, chlorophenyl-H and SO_2NH_2), 7.76 (s, 1H, =CH), 8.15, 8.42 (2d, $J = 9.0$ Hz,

each 2H, benzene sulfonamide $C_{3,5}$–H and $C_{2,6}$–H), 8.49 (s, 1H, pyrazole C_3–H), 10.53, 12.19 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 104.84$ (pyrazole C_4), 118.13 (thiazolidinone C_5), 128.80, 129.43, 132.43, 133.15, 135.33, 136.12, 137.46, 147.84 (chlorophenyl-C and benzenesulfonamide-C), 148.64 (pyrazole C_3), 149.22 (=CH), 150.69 (pyrazole C_5), 158.28 (thiazolidinone C_2), 166.15, 171.23 (C=O) ppm; IR (KBr): $\bar{\nu} = 3347$, 3216 (NH), 3097, 2927 (CH), 1695 (C=O), 1619 (C=N), 1596, 1546, 1495 (C=C), 1334, 1160 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 519 ($[M + 2]^+$, 31.5), 517 (M^+, 91.2), 413 (203.0).

4-[5-Amino-4-[[(2E)-2-[(5Z)-5-[(1,3-diphenyl-1H-pyrazol-4-yl)methylene]-4-oxo-1,3-thiazolidin-2-ylidene]hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**15a**, $C_{29}H_{23}N_9O_4S_2$)
Yellow solid (0.7 g, 56 %); m.p.: 301–302 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 6.68$ (s, 2H, NH_2, D_2O exchangeable), 7.40–7.62 (m, 11H, *N*-phenyl–H, phenyl $C_{3,4,5}$–H, SO_2NH_2 and =CH), 7.66 (d, $J = 8.6$ Hz, 2H, benzenesulfonamide $C_{3,5}$–H), 7.96 (d, $J = 8.7$ Hz, 2H, phenyl $C_{2,6}$–H), 7.99 (d, $J = 8.6$ Hz, 2H, benzenesulfonamide $C_{2,6}$–H), 8.10 (s, 1H, pyrazole C_3–H), 8.73 (s, 1H, pyrazole C_5–H), 10.70, 12.54 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 94.65$, 115.76 (pyrazole C_4), 119.75 (thiazolidinone C_5), 120.48, 123.32, 123.62, 124.13, 127.78, 128.16, 129.66, 128.58, 130.45, 130.59, 130.64, 134.42, 139.85, 141.64, 142.79, 150.16, 152.18, 152.48 (Ar–C), 162.76, 172.69 (C=O) ppm; IR (KBr): $\bar{\nu} = 3442$, 3339, 3230 (NH), 3099, 3059, 2948 (CH), 1690, 1660 (C=O), 1616 (C=N), 1545, 1501, 1435 (C=C), 1336, 1163 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 625 (M^+, 45), 88 (100.0).

4-[5-Amino-4-[[(2E)-2-[(5Z)-5-[[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene]-4-oxo-1,3-thiazolidin-2-ylidene]hydrazinyl]carbonyl]-1H-pyrazol-1-yl]benzenesulfonamide (**15b**, $C_{29}H_{22}ClN_9O_4S_2$)
Yellow solid (0.66 g, 50 %); m.p.: 296–297 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 6.68$ (s, 2H, NH_2, D_2O exchangeable), 7.39–7.98 (m, 16H, *N*-phenyl–H, chlorophenyl–H, SO_2NH_2, = CH and benzenesulfonamide-H), 8.11 (s, 1H, pyrazole C_3–H), 8.72 (s, 1H, pyrazole C_5–H), 10.53, 12.40 (2 s, each 1H, 2 NH, D_2O exchangeable) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 96.55$, 116.28 (pyrazole C_4), 119.72 (thiazolidinone C_5), 119.92, 123.46, 123.52, 127.47, 127.90, 128.45, 129.24, 129.50, 130.08, 130.74, 130.79, 134.27, 139.49, 141.02, 142.76, 150.76, 152.31, 152.42 (Ar–C), 162.78, 172.9 (C=O) ppm; IR (KBr): $\bar{\nu} = 3393$, 3240 (NH), 3050, 2922 (CH), 1696 (C=O), 1613 (C=N), 1528, 1504, 1433 (C=C), 1331, 1158 (SO_2) cm^{-1}; MS (70 eV) *m/z* (%) = 661 ($[M + 2]^+$, 16), 659 (M^+, 49.4), 88 (100.0).

Anti-inflammatory (AI) activity

Formalin-induced paw edema bioassay
Male albino rats weighing 180–200 g were used throughout the assay. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups each of six rats. One group of six rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 20 mg/kg body weight po). A solution of formalin (2 %, 0.1 cm^3) was injected into the subplantar region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound (at a dose level of 20 mg/kg body weight). The paw volume (cm^3) was measured by means of water plethysmometer and remeasured again 1, 2, and 4 h after administration of formalin. 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 is the volume of edema at a specific time interval and V_0 is the volume of edema at zero time interval.

Determination of effective dose $_{50}$ (ED_{50})
The selected compounds were further tested at 5, 10, 20, 40, and 50 mg/kg body weight and the ED_{50} was determined by measuring the inhibition of edema volume 4 h after formalin injection.

Analgesic activity

Analgesic activity was determined using the tail withdrawal response to immersion of rat tail in water at 55 °C according to the procedure described by Janssen et al. [28]. Male albino rats weighing 120–150 g were used throughout this assay. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups each of six rats. One group of six rats was kept as a control and another group received the standard drug diclofenac sodium (at a dose of 20 mg/kg body weight po). The tested compounds were administrated orally at a dose of 20 mg/kg. The recorded values were the average of six administrations ±SE and the percentage increase of the reaction time (after 1–4 h time intervals) was calculated in comparison with the basal values according to the following equation:

$$\%\,\text{increase of the reaction time} = (T_t/T_0)_{\text{tested compound}} - 1 \times 100,$$

where T_t is the reaction time at a specific time interval and T_0 is the reaction time at zero time interval.

Ulcerogenic activity

Male albino rats (180–200 g) were divided into groups of six animals each and fasted for 24 h prior to administration of the test compounds. Water was given ad libitum. The control group received 1 % gum acacia orally. Other groups received diclofenac sodium or the test compounds orally in two equal doses at 0 and 2 h for three successive intervals at a dose of 250 mg/kg per day. The animals were killed by diethyl ether 6 h after the last dose and their stomach was removed, opened along the greater curvature, and examined (using a dissecting microscope) for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosions, or ulcers. The extent of lesions, their number and size were rated on a scale from 0 to 3.

Acute toxicity

Twelve groups of rats (180–200 g), each consisting of six animals, were used in this test. The animals were fasted for 24 h prior to administration of the test compounds. The compounds were given orally in graded doses of 0.1–0.25 g/kg body weight, po. The compounds were screened at graded doses for their acute lethal doses (ALD_{50}) and the mortalities were recorded at each dose level after 24 h.

Animal care and all experimental procedures used in this study were performed in accordance with the regulations and guidelines stipulated by the Institutional Animal Care and Use Committee (ACUC) at Alexandria University, Egypt.

Statistical analysis of data

The data obtained are presented as mean ± SE of the mean. The concentration-dependent effects of various drugs in vitro were evaluated statistically by the randomized block design analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple comparison test. The difference in results was considered significant when $p < 0.05$.

In vitro COX study

The inhibitory COX activity of the tested compounds and the reference was assayed using Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111, Cayman chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions. Diclofenac and celecoxib were used as reference standards in the study.

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