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Original article

Synthesis of novel 1,3,4-trisubstituted pyrazoles as anti-inflammatory and analgesic agents

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ABSTRACT

Some novel 1,3,4-trisubstituted pyrazoles were synthesized and screened for their anti-inflammatory and analgesic activities as well as their ulcerogenic liability. They showed anti-inflammatory and analgesic activities with better GIT tolerance than the standard drug phenylbutazone. In addition, IC_{50} values for **5e** and **8e** were recorded. Compound **5e** was found to be the most active one as anti-inflammatory and analgesic agent. On the other hand, COX-1/COX-2 isozyme selectivity was also done which showed equal inhibition to both isoforms.

1. Introduction

Design of non-steroidal anti-inflammatory drugs (NSAIDs) with enhanced safety profile is still a challenge for the pharmaceutical industry. In spite of the great safety of known NSAIDs over the steroidal derivatives, they still have some side effects like development of peptic and duodenal ulcer in addition to some kidney and liver malfunctions on long term use [1].

The acceptable mechanism of action of NSAIDs is by the inhibition of the biosynthesis of prostaglandins through inhibition of the key enzyme known as prostaglandin cyclooxygenase (COX) [2]. COX exists in two isoforms namely COX-1 and COX-2. COX-1 is a constitutive enzyme and responsible for the production of cyclo-protective prostaglandin in GIT and proaggregatory thromboxane in blood platelets, while COX-2 is an inducible enzyme which induces in response to the release of several proinflammatory mediators [3–5]. Most NSAIDs inhibit both COX-1 and COX-2 but with variable degrees of selectivity. Selective COX-2 inhibitors may eliminate side effects that are associated with NSAIDs due to COX-1 inhibition [6–8]. However, selective COX-2 inhibitors are found to posses cardiovascular side effects [9].

Pyrazole derivatives as various coxibs showed potent anti-inflammatory activity with low GIT toxicity [10,11]. Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib and SC-558 (Fig. 1) which belong to diaryl pyrazoles containing sulfonamide moiety.

Consequently, the present investigation describes the synthesis of certain 1,3-diaryl pyrazoles, position 1 of the pyrazole ring was substituted either with benzenesulfonamide or 4-chlorophenyl group, since these two substituents recorded to exhibit good anti-inflammatory activity [12–18]. Position 3 carries either *p*-tolyl or 4-methoxy phenyl substituent. Moreover, position 4 of the pyrazole ring was substituted with a variety of functionality as azomethine, chalcone or substituted pyrrole derivatives as it was reported that these derivatives have a promising effect in potentiating the anti-inflammatory activity [15,19–25]. All the synthesized compounds were subjected to anti-inflammatory testing. The ulcerogenic activity and acute toxicity profile of the most active compounds and their selectivity to inhibit COX-1 and COX-2 isozymes by *in vitro* COX inhibition assay were determined. In addition, the analgesic activity of representative examples was also carried out.

2. Results and discussion

2.1. Chemistry

The target compounds were synthesized according to the steps outlined in Schemes 1 and 2. The key intermediates 1-(4-substituted phenyl)-2-(1-(4-substituted phenyl)ethylidene)

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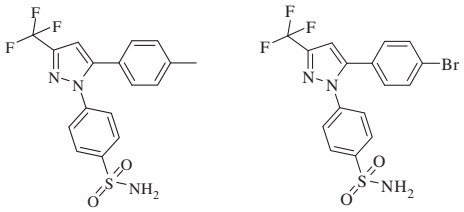
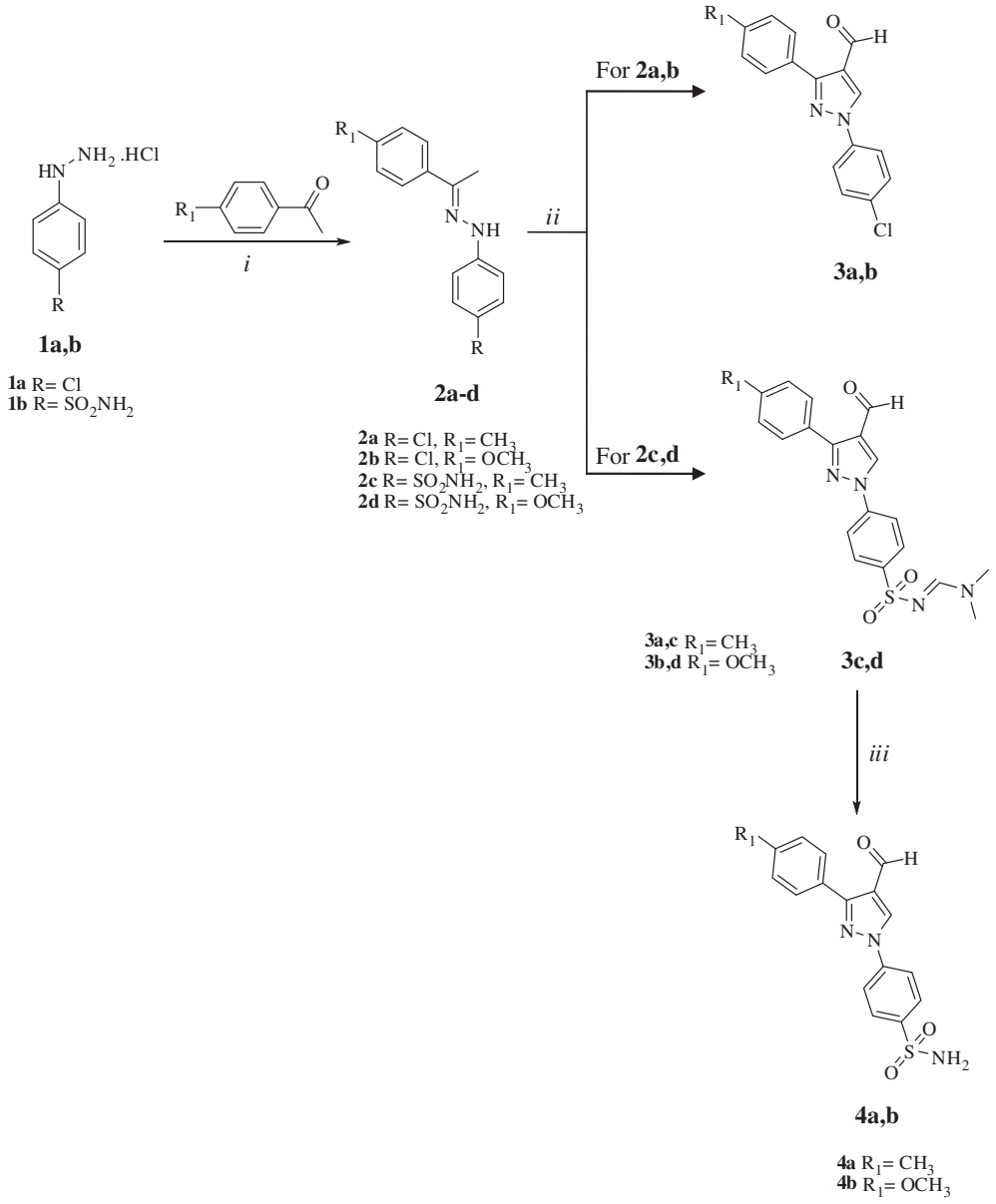


Celecoxib SC-558

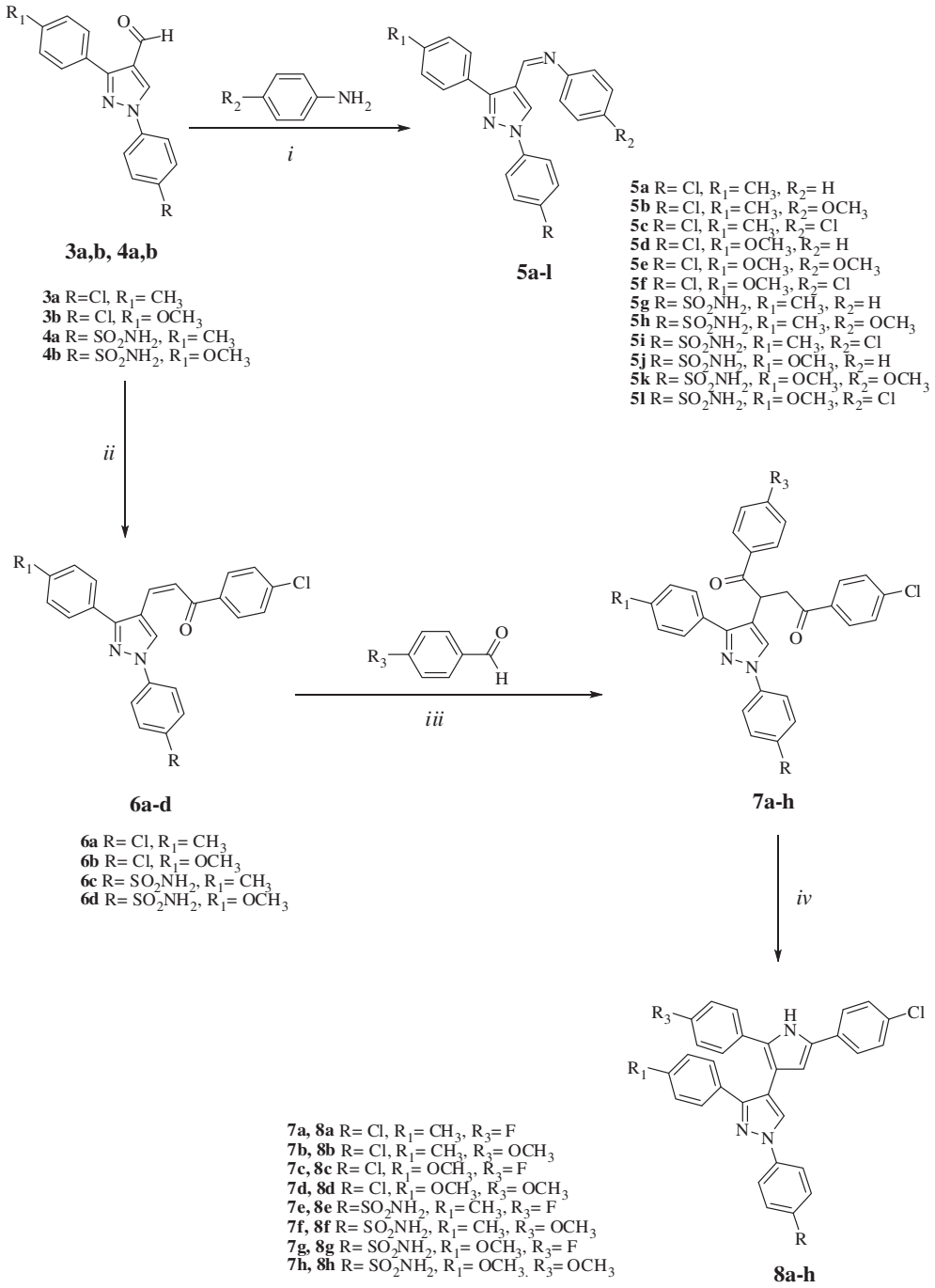
Fig. 1. Structure of COX-2 selective agents.

hydrazines **2a**–**d** were synthesized by condensation of the commercially available 4-chlorophenylhydrazine hydrochloride **1a** or the synthesized sulfamoyl counterpart **1b** [26] with 4-substituted acetophenones. Treatment of the hydrazone derivatives **2a**,**b** with Vilsmeier–Haack [27] reagent (DMF–$POCl_3$) in three equivalents led to corresponding 4-formylpyrazole derivatives **3a** [28] and the newly synthesized **3b**. Reaction of the hydrazone derivatives **2c**,**d** with Vilsmeier–Haack reagent afforded the corresponding 4-formylpyrazole derivatives with protected sulfonamido group **3c**,**d** [13,29]. Deprotection was accomplished using methanolic solution of NaOH with THF as co-solvent [29]. Bekhit et al. [13] reported that acidic conditions could be also used for the deprotection of the sulfonamido group, but the basic conditions reported by Sharma et al. [29] applied here gave much better yield (Scheme 1).

As shown in Scheme 2, the 4-formylpyrazoles **3a**,**b** and **4a**,**b** were functionalized by a classical condensation reaction with substituted anilines in refluxed ethanol with traces of glacial acetic acid to give the corresponding azomethine derivatives **5a**–**l**. The ^{1}H NMR spectra of **5a**–**l** showed the appearance of a signal at δ 8.49–8.64 ppm corresponding to azomethine proton (N=C*H*) and the disappearance of the signal attributed to (C*H* of aldehyde). Also, ^{13}C NMR spectrum of **5e** revealed the disappearance of the signal corresponding to (HC=O).



Scheme 1. Synthesis of the target compounds **1**–**4**: (i) EtOH, glacial acetic, r.t. (ii) $POCl_3$, DMF, reflux. (iii) MeOH, THF, NaOH, r.t.



Scheme 2. Synthesis of the target compounds **5**–**8**: (i) EtOH, glacial acetic, reflux. (ii) *p*-chloroacetophenone, NaOH, EtOH, r.t. (iii) DMF, r.t. (iv) NH_4-acetate, acetic acid, reflux.

On the other hand, Claisen–Schmidt [30,31] condensation between **3a**,**b** or **4a**,**b** and 4-chloroacetophenone afforded the corresponding chalcones **6a**–**d**. ^{1}H NMR spectra of **6a**–**d** showed the appearance of two doublets corresponding to olefinic protons of α,β-unsaturated ketone (*H*C═C*H*–C═O) at δ 7.01–7.89 ppm and the disappearance of the signal corresponding to (C*H* of aldehyde). Also the mass spectra of **6a**,**b** revealed the appearance of their molecular ion peaks along with isotopes $(M + 2)^+$, $(M + 4)^+$ that indicate the presence of two chlorine atoms. Addition reaction of **6a**–**d** with anisaldehyde or 4-fluorobenzaldehyde under Stetter [32] reaction conditions using dimethylformamide in the presence of potassium cyanide as catalyst afforded compounds **7a**–**h**. Their ^{1}H NMR spectra showed the appearance of aliphatic protons of (–C*H*$_2$–C*H*–) as a prominent ABX system, with protons Ha, Hb and Hx seen as doublets of doublets at δ 3.23–3.45, 3.43–3.61 and 4.19–4.70 ppm, respectively. **7a**–**h** were cyclized using ammonium acetate in acetic acid under Paal–Knorr [33,34] reaction conditions to yield the corresponding 1-(4-substitutedphenyl)-4-(5-(4-chlorophenyl)-2-(4-substitutedphenyl)-1*H*-pyrrol-3-yl)-3-(4-substitutedphenyl)-1*H*-pyrazoles **8a**–**h**. Their IR spectra revealed the appearance of a band at 3383–3417 cm^{-1} corresponding to (NH) and the disappearance of any band corresponding to (C═O), ^{1}H NMR spectra showed the appearance of an exchangeable signal of (N*H*) at δ 5.00–5.90 ppm and the disappearance of any doublets of doublets

corresponding to the aliphatic protons of (−CH_2−CH), also ^{13}C NMR spectrum of **8d** revealed the disappearance of any signal corresponding to (C=O) (Scheme 2).

2.2. Pharmacological screening

2.2.1. Anti-inflammatory activity

All the targeted compounds were evaluated for their anti-inflammatory activity via carrageenan-induced rat paw edema method reported by Winter et al. [35] using phenylbutazone as reference drug. The results were recorded in Table 1. IC_{50} values of the active compounds **5e** and **8e** and the reference drug phenylbutazone were calculated and recorded in Table 2.

Results in Table 1 revealed that all the tested compounds showed anti-inflammatory activity. After 2 h eleven compounds exhibited better activity than phenylbutazone. While, after 3 h only five compounds gave better activity than phenylbutazone.

2.2.2. Structure–activity relationship

Considering the anti-inflammatory activity of the pyrazole carboxyaldehydes **3a**,**b** and **4a**,**b**, it was noticed that the 1-(benzenesulfonamide) derivatives **4a**,**b** showed higher activity than their 1-(4-chlorophenyl) congeners **3a**,**b**. However, on the formation of the azomethine derivatives **5a**–**l** the case was different; compounds **5d**–**f** bearing 4-chlorophenyl pharmacophore exhibited higher activity than 1-(benzenesulfonamide) analogs **5j**–**l** except for benzenesulfonamide azomethine derivatives **5g**,**i** which possessed higher activity than their 4-chlorophenyl analogs **5a**,**c**.

Also, the effect of the nature of substituent at the position-3 of the pyrazole nucleus was studied. This position was substituted either by *p*-tolyl or 4-methoxy phenyl moiety; in the pyrazole carboxyaldehydes **3a**,**b** and **4a**,**b** the 3-(4-methoxy phenyl) derivatives **3b** and **4b** exhibited higher activity than their *p*-tolyl congeners **3a** and **4a**. For the 1-(4-chlorophenyl) derivatives of the azomethine series **5a**–**f**, improved activity was observed by the 3-(4-methoxy phenyl) substituted compounds **5d**–**f** than their *p*-tolyl analogs **5a**–**c**. On the other hand, in the benzenesulfonamide azomethine series **5k** was the only derivative which showed improved activity than its *p*-tolyl analog **5h**.

Moreover, the nature of the aryl substituent attached to the azomethine group has an observable effect on the activity, as shown in compounds **5b**,**e**,**k** bearing 4-electron donating substituent (methoxy group) that exhibited improved activity than their congeners with 4-electron withdrawing substituent (chloro group) **5c**,**f**,**l** or with unsubstituted phenyl analogs **5a**,**d**,**j**. On the contrary, compound **5i** was the only one bearing 4-choloro substituent and has superior activity than its 4-methoxy or unsubstituted phenyl congeners **5h**,**g**, respectively.

Chalcones carrying 1-(4-chlorophenyl) pharmacophore **6a**,**b** exhibited greater activity than their 1-(benzenesulfonamide) counterparts **6c**,**d**. Also, the 3-(4-methoxyphenyl) derivatives **6b**,**d** were more active than the 3-(*p*-tolyl) derivatives **6a**,**c**. While, in the series of **7a**–**h**, 1-(benzenesulfonamide) compounds **7e**–**h** showed much greater activity than their 1-(4-chlorophenyl) analogs **7a**–**d**. Rigidification of **7a**–**h** by incorporation of additional pyrrole ring as shown in compounds **8a**–**h** resulted in improvement of activity except for compounds **8b**,**f**,**g** which showed decreased activity compared with **7b**,**f**,**g**. Moreover, 4-substitution of phenyl moiety attached to pyrrole ring by electron withdrawing group as fluoro group in compounds **8a**,**c**,**e**,**g** found to exhibit a fruitful effect on activity than with electron donating one as methoxy analogs **8b**,**d**,**f**,**h**.

2.2.3. Analgesic activity

Compounds that exhibited good anti-inflammatory activity and phenylbutazone were screened for their analgesic activity using the reported method of *p*-benzoquinone-induced writhing in mice by Okun et al. [36] It was observed from the results in Table 3 that, all the tested compounds showed analgesic activity. Moreover, compound **5e** that showed the best anti-inflammatory activity also found to be the most active one as analgesic.

Table 1
Anti-inflammatory effect and percentage inhibition of phenylbutazone, and synthesized compounds on carrageenan induced edema of the hind paw in rats ($n = 5$).

Cpd. NO.	Dose mmol/kg	Edema (mm) ± SEM		% inhibition	
		2 h	3 h	2 h	3 h
Control	0	3.24 ± 0.39	3.60 ± 0.24	0	0
Phenylbutazone	0.32	1.38 ± 0.09***	1.06 ± 0.19***	57.28	70.55
3a	0.32	1.62 ± 0.18***	1.58 ± 0.12***	49.90	56.00
3b	0.32	1.19 ± 0.11***	1.53 ± 0.22***	63.11	57.58
4a	0.32	1.35 ± 0.15***	1.15 ± 0.12***	58.40	68.17
4b	0.32	0.55 ± 0.09***	0.66 ± 0.11***	82.96	81.55
5a	0.32	1.94 ± 0.04***	1.82 ± 0.16***	40.06	49.33
5b	0.32	1.23 ± 0.16***	1.84 ± 0.13***	62.04	48.89
5c	0.32	1.68 ± 0.19***	1.83 ± 0.26***	48.27	49.17
5d	0.32	1.40 ± 0.09***	0.99 ± 0.15***	57.19	72.58
5e	0.32	0.99 ± 0.17***	0.64 ± 0.15***	69.23	82.22
5f	0.32	0.98 ± 0.11***	0.99 ± 0.17***	69.60	72.60
5g	0.32	1.36 ± 0.20***	1.54 ± 0.21***	58.08	57.11
5h	0.32	1.66 ± 0.09***	1.58 ± 0.16***	48.76	56.19
5i	0.32	1.00 ± 0.12***	0.78 ± 0.14***	69.10	78.40
5j	0.32	1.99 ± 0.09**	1.61 ± 0.09***	38.45	55.40
5k	0.32	1.11 ± 0.15***	1.13 ± 0.14***	65.80	68.50
5l	0.32	1.19 ± 0.19***	1.34 ± 0.22***	63.04	62.78
6a	0.32	1.65 ± 0.17***	1.39 ± 0.25***	49.19	61.40
6b	0.32	1.50 ± 0.16***	1.21 ± 0.26***	53.70	66.39
6c	0.32	1.51 ± 0.13***	1.90 ± 0.13***	53.33	47.60
6d	0.32	1.44 ± 0.29***	1.58 ± 0.14***	55.56	55.97
7a	0.32	2.40 ± 0.32	2.90 ± 0.29	25.90	19.44
7b	0.32	2.72 ± 0.17	2.74 ± 0.07	16.05	23.89
7c	0.32	2.59 ± 0.26	2.24 ± 0.27**	19.80	37.83
7d	0.32	3.47 ± 0.11	3.25 ± 0.22	0	9.61
7e	0.32	1.51 ± 0.06***	1.58 ± 0.14***	53.46	56.22
7f	0.32	1.49 ± 0.18***	1.40 ± 0.20***	53.83	61.11
7g	0.32	0.90 ± 0.12***	1.15 ± 0.30***	72.22	68.06
7h	0.32	2.24 ± 0.24	2.79 ± 0.18	30.80	22.50
8a	0.32	2.00 ± 0.34*	2.84 ± 0.29	38.27	20.97
8b	0.32	2.94 ± 0.13	2.83 ± 0.17	9.38	21.33
8c	0.32	1.51 ± 0.26***	1.37 ± 0.16***	53.70	61.89
8d	0.32	2.90 ± 0.23	2.85 ± 0.11	10.50	20.72
8e	0.32	0.79 ± 12***	1.19 ± 0.09***	75.37	66.70
8f	0.32	1.63 ± 0.22***	2.37 ± 0.19**	49.69	34.17
8g	0.32	1.43 ± 0.15***	1.59 ± 0.15***	55.70	55.90
8h	0.32	1.68 ± 0.25***	2.57 ± 0.28	48.15	28.60

Statistical analysis was carried out by one-way ANOVA test.
*Significance difference from the control value at $p < 0.05$.
**Significance difference from the control value at $p < 0.01$.
***Significance difference from the control value at $p < 0.001$.

Table 2
IC_{50} values of compounds **5e** and **8e** and the reference drug phenylbutazone.

Cpd. No.	Dose (mmol/kg)	% inhibition	IC_{50} (mmol/kg)
Phenylbutazone	0.08	20.00	0.22
	0.16	42.10	
	0.32	70.55	
5e	0.08	37.50	0.20
	0.16	43.00	
	0.32	82.22	
8e	0.08	14.00	0.21
	0.16	43.60	
	0.32	66.70	

Table 3
Analgesic activity of phenylbutazone and 17 synthesized compounds in mice.

Cpd. No.	Dose mmol/kg	No. of animals	No. of protected animals	% protection
Control	0	6	0	0
Phenylbutazone	0.32	6	2	33.33
3b	0.32	6	2	33.33
4a	0.32	6	1	16.67
4b	0.32	6	1	16.67
5b	0.32	6	1	16.67
5d	0.32	6	1	16.67
5e	0.32	6	4	66.67
5f	0.32	6	2	33.33
5i	0.32	6	2	33.33
5k	0.32	6	2	33.33
5l	0.32	6	3	50
7e	0.32	6	3	50
7f	0.32	6	3	50
7g	0.32	6	2	33.33
8a	0.32	6	2	33.33
8c	0.32	6	2	33.33
8e	0.32	6	1	16.67
8f	0.32	6	3	50

Table 4
Ulcerogenic effect of phenylbutazone and 9 synthesized compounds in rats.

Cpd. No.	Dose mmol/kg	No. of animals	% incidence divided by 10	Average no. of ulcer	Average severity	Ulcer index
Control	0	5	0	0	0	0
Phenylbutazone	0.32	5	10	19.0	1.20	30.20
4a	0.32	5	6	2.6	1.00	9.60
4b	0.32	5	8	2.2	1.00	11.20
5e	0.32	5	10	3.2	1.12	14.32
5f	0.32	5	4	0.4	1.00	5.40
5i	0.32	5	4	0.8	1.00	5.80
5k	0.32	5	10	5.4	1.00	16.40
8a	0.32	5	8	4.0	1.00	13.00
8c	0.32	5	8	3.0	1.00	12.00
8e	0.32	5	10	4	1	15.00

2.2.4. Ulcerogenic effect

The ulcerogenic effect of nine active compounds and phenylbutazone was evaluated by the reported method of Meshali et al. [37], and the ulcer index was calculated according to the method of Robert et al. [38] and recorded in Table 4. It was observed that all the tested compounds showed lower ulcer indices than phenylbutazone. Moreover, concerning the tested Schiff's bases it was noticed that the chloro derivatives **5f**,**i** showed less ulcerogenic effects than their methoxy analogs **5e**,**k**.

2.2.5. Acute toxicity

LD_{50} of some representative compounds **5e**,**k**,**l**, and **8e** was determined using Finney's method [39]. Results revealed that LD_{50} of compounds **5e**,**k**, and **8e** was 3.25 mmol/kg body weight, while that of compound **5l** was 2.44 mmol/kg body weight which indicated a high safety margin of the tested compounds.

Table 5
COX-1/COX-2 percentage inhibition ratio of compounds **5e**, **5f**, **8e** and indomethacin.

Cpd. NO.	COX-1/COX-2
Indomethacin	3.94
5e	1.17
5f	0.95
8e	0.87

2.2.6. In vitro COX inhibition assay

Compounds **5e**,**f**, **8e**, and indomethacin were evaluated for their selectivity to inhibit COX-1 and/or COX-2 isozymes using Cayman's COX-Activity Assay kit according to the manufacturer's instruction [40]. The ratio of percentage inhibition of COX-1/COX-2 was calculated and recorded in Table 5. Results revealed that the tested compounds were non-selective as they have almost equal inhibitory activity on both isoforms of COX enzyme.

3. Conclusion

From this study, we conclude that 1-(4-chlorophenyl)pyrazole and pyrazolylbenzenesulfonamide derivatives could be considered to be useful building blocks for future research to the synthesis of potential anti-inflammatory and analgesic agents with minimal side effects. Pharmacological screening and biological evaluation revealed that all the tested compounds have good anti-inflammatory activity. The newly synthesized compounds **5e**,**f**, and **8e** showed high anti-inflammatory activity and good analgesic activity. These compounds were non-selective toward COX-1 or COX-2 but they exhibited better GIT tolerance than the reference drug phenylbutazone.

4. Experimental

All chemicals were purchased from VWR International Merck, Germany or Sigma–Aldrich and used without further purification. Compound (4-chlorophenyl)hydrazine hydrochloride (**1a**) was purchased from Sigma–Aldrich. Melting Points were carried out by open capillary tube method using Stuart SMP3 Melting Point apparatus and they are uncorrected. Elemental Microanalysis was carried out at the Micro Analytical Center, Cairo University or at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded on Shimadzu Infrared spectrometer IR Affinity-1 (FTIR-8400S-Kyoto-Japan), and expressed in wave number (cm^{-1}), using potassium bromide discs. ^1H NMR and ^{13}C NMR Spectra were recorded on a Varian Mercury VX-300 MHz, ^{13}C, 70 MHz NMR spectrometer, or Jeol-ECA500, 500 MHz Japan, ^{13}C, 125 MHz NMR spectrometer, the spectra were run at 300 MHz or 500 MHz in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts were expressed in δ units and were related to that of the solvents. As for the proton magnetic resonance, D_2O was carried out for NH and OH exchangeable protons. Mass Spectra were recorded using Shimadzu Gas Chromatograph Mass spectrometer-Qp 2010 plus (Japan) or Jeol JMS-AX 500 mass spectrometer (Japan). All the reactions were followed by TLC using silica gel F254 plates (Merck), using chloroform: methanol 9:1 or chloroform as eluting system and were visualized by UV-lamp. Compounds **1b** [26], **3a** [28], **3c** [13,29], **3d** [29], **4a** [13,29], **4b** [29] were prepared according to reported methods, **2a** [28], **2c** [13,29] and **2d** [29] were prepared by modified procedure from the reported method.

4.1. Chemistry

4.1.1. General procedure for the synthesis of compounds (*2a–d*)

A mixture of **1a**,**b** (10 mmol) in glacial acetic acid (1 mL) was added to a solution of 4-substituted acetophenone (10 mmol) in ethanol (30 mL). Then, the reaction mixture was stirred at room temperature for 24 h, concentrated under reduced pressure and poured onto ice water. The solid formed was collected, washed with water, dried and recrystallized from ethanol.

*4.1.1.1. 1-(4-Chlorophenyl)-2-(1-p-tolylethylidene)hydrazine (**2a**).* Yield 73%; as oil; IR (KBr, cm^{-1}): 3352 (NH), 3074 (CH aromatic), 2916, 2854 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.42 (s, 3H,

CH_3), 2.58 (s, 3H, =C–CH_3), 4.73 (s, ex, 1H, NH), 6.77–7.87 (m, 8H, aromatic H); EIMS, *m*/*z*: 260 $(M + 2)^+$, 258 (M^+); Anal. Calcd. For $C_{15}H_{15}ClN_2$ (258.74): C, 69.63; H, 5.84; N, 10.83. Found: C, 69.74; H, 5.92; N, 10.91.

*4.1.1.2. 1-(4-Chlorophenyl)-2-(1-(4-methoxyphenyl)ethylidene)hydrazine (**2b**).* Yield 66%; m.p. 128–130 °C; IR (KBr, cm^{-1}): 3356 (NH), 3000 (CH aromatic), 2962, 2935 (CH aliphatic); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.55 (s, 3H, =C–CH_3), 3.84 (s, 3H, OCH_3), 4.90 (s, ex, 1H, NH), 6.76–7.94 (m, 8H, aromatic H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 26.4, 55.5, 114.3, 122.4, 124.2, 127.0, 129.1, 130.2, 130.8, 142.4, 159.8, 163.7; EIMS, *m*/*z*: 276 $(M + 2)^+$, 274 (M^+); Anal. Calcd. For $C_{15}H_{15}ClN_2O$ (274.74): C, 65.57; H, 5.50; N, 10.20. Found: C, 65.62; H, 5.48; N, 10.47.

*4.1.1.3. 4-(2-(1-p-Tolylethylidene)hydrazinyl)benzenesulfonamide (**2c**).* Yield 52%; m.p. 226–227 °C; IR (KBr, cm^{-1}): 3363, 3336 (NH_2), 3267 (NH), 3050 (CH aromatic), 2950, 2830 (CH aliphatic), 1319, 1145 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.26 (s, 3H, CH_3), 2.57 (s, 3H, =C–CH_3), 4.65 (s, ex, 1H, NH), 7.25 (s, ex, 2H, NH_2), 7.19–7.82 (m, 8H, aromatic H); EIMS, *m*/*z*: 303 (M^+); Anal. Calcd. For $C_{15}H_{17}N_3O_2S$ (303.37): C, 59.38; H, 5.65; N, 13.85. Found: C, 59.57; H, 5.08; N, 13.46.

*4.1.1.4. 4-(2-(1-(4-Methoxyphenyl)ethylidene)hydrazinyl)benzenesulfonamide (**2d**).* Yield 78%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3353, 3321 (NH_2, NH), 3070, 3012 (CH aromatic), 2978 (CH aliphatic), 1319, 1145 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.25 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.65 (s, ex, 1H, NH), 7.26 (s, ex, 2H, NH_2), 6.92–7.82 (m, 8H, aromatic H); EIMS, *m*/*z*: 319 (M^+); Anal. Calcd. For $C_{15}H_{17}N_3O_3S$ (319.37): C, 56.41; H, 5.37; N, 13.16. Found: C, 56.69; H, 5.58; N, 13.26.

*4.1.2. General procedure for the synthesis of compounds (**3a**,**b**)*

A mixture of DMF (2.58 g, 35.30 mmol) and $POCl_3$ (5.40 g, 35.30 mmol) was cooled at 0 °C before being stirred at that temperature. A solution of **2a**–**d** (11.76 mmol) in DMF (3 mL) was added drop wise to the Vilsmeier–Haack reagent, warmed to room temperature, and heated at 70–80 °C for 5 h. After cooling to room temperature, the mixture was basified with cold saturated K_2CO_3 solution. The precipitate was filtered, washed with water and crystallized from ethanol.

*4.1.2.1. 1-(4-Chlorophenyl)-3-p-tolyl-1H-pyrazole-4-carbaldehyde (**3a**).* Yield 90%; m.p. 132–133 °C; IR (KBr, cm^{-1}): 3050 (CH aromatic), 2920 (CH aliphatic), 2870, 2781 (CH aldehyde), 1670 (C=O aldehyde); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 7.27–7.77 (m, 8H, aromatic H), 8.51 (s, 1H, CH of pyrazole), 10.05 (s, 1H, CHO); EIMS, *m*/*z*: 298 $(M + 2)^+$, 296 (M^+); Anal. Calcd. For $C_{17}H_{13}ClN_2O$ (296.75): C, 68.81; H, 4.42; N; 9.44. Found: C, 68.94; H, 4.48; N, 9.63.

*4.1.2.2. 1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde (**3b**).* Yield 92%; m.p. 142–143 °C; IR (KBr, cm^{-1}): 3070 (CH aromatic), 2958, 2931 (CH aliphatic), 2831, 2781 (CH aldehyde), 1674 (C=O aldehyde); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.87 (s, 3H, OCH_3), 7.01–7.79 (m, 8H, aromatic H), 8.47 (s, 1H, CH of pyrazole), 10.03 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 70 MHz): δ 55.3, 114.1, 120.7, 122.5, 123.5, 129.7, 130.1, 131.0, 133.4, 137.5, 154.5, 160.6, 184.8; EIMS, *m*/*z*: 314 $(M + 2)^+$, 312 (M^+); Anal. Calcd. For $C_{17}H_{13}ClN_2O_2$ (312.75): C, 65.29; H, 4.19; N, 8.96. Found: C, 65.32; H, 4.16; N, 9.33.

*4.1.3. General procedure for the synthesis of compounds (**5a**–**l**)*

To a hot solution of **3a**,**b** or **4a**,**b** (2 mmol) in ethanol (15 mL) glacial acetic acid (0.2 mL) was added followed by aromatic amines (aniline, 4-anisidine or 4-chloroaniline) (2 mmol), the reaction mixture was refluxed for 12–15 h. Then after cooling to room temperature, the solid formed was collected by filtration, washed with water and recrystallized from ethanol.

*4.1.3.1. N-((1-(4-Chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)methylene)aniline (**5a**).* Yield 71%; m.p. 197–198 °C; IR (KBr, cm^{-1}): 3030 (CH aromatic), 2920, 2854 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 6.68–7.83 (m, 14H, aromatic H+ CH pyrazole), 8.53 (s, 1H, HC=N); EIMS, *m*/*z*: 373 $(M + 2)^+$, 371 (M^+); Anal. Calcd. For $C_{23}H_{18}ClN_3$ (371.86): C, 74.29; H, 4.88; N, 11.30. Found: C, 74.38; H, 4.97; N, 11.66.

*4.1.3.2. N-((1-(4-Chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)methylene)-4-methoxyaniline (**5b**).* Yield 45%; m.p. 152–153 °C; IR (KBr, cm^{-1}): 3101, 3059 (CH aromatic), 2920, 2850 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 6.91–7.82 (m, 13H, aromatic H+ CH of pyrazole), 8.51 (s, 1H, HC=N); EIMS, *m*/*z*: 403 $(M + 2)^+$, 401 (M^+); Anal. Calcd. For $C_{24}H_{20}ClN_3O$ (401.88): C, 71.73; H, 5.02; N, 10.46. Found: C, 71.81; H, 5.11; N, 10.73.

*4.1.3.3. 4-Chloro-N-((1-(4-chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)methylene)aniline (**5c**).* Yield 55%; m.p. 151–153 °C; IR (KBr, cm^{-1}): 3036 (CH aromatic), 2920, 2850 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 6.59–7.84 (m, 13H, aromatic H+ CH of pyrazole), 8.51 (s, 1H, HC=N); EIMS, *m*/*z*: 409 $(M + 4)^+$, 407 $(M + 2)^+$, 405 (M^+); Anal. Calcd. For $C_{23}H_{17}Cl_2N_3$ (406.30): C, 67.99; H, 4.22; N, 10.34. Found: C, 68.08; H, 4.31; N, 10.59.

*4.1.3.4. N-((1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene)aniline (**5d**).* Yield 63%; m.p. 159–160 °C; IR (KBr, cm^{-1}): 3070, 3000 (CH aromatic), 2924, 2904 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 3H, OCH_3), 6.68–7.83 (m, 14H, aromatic H+ CH of pyrazole), 8.50 (s, 1H, HC=N); EIMS, *m*/*z*: 389 $(M + 2)^+$, 387 (M^+); Anal. Calcd. For $C_{23}H_{18}ClN_3O$ (387.86): C, 71.22; H, 4.68; N, 10.83. Found: C, 71.31; H, 4.77; N, 11.18.

*4.1.3.5. N-((1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene)-4-methoxyaniline (**5e**).* Yield 60%; m.p. 103–105 °C; IR (KBr, cm^{-1}): 3070, 3008 (CH aromatic), 2966, 2916 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.91–7.78 (m, 12H, aromatic H), 8.50 (s, 1H, CH of pyrazole), 8.61 (s, 1H, HC=N); ^{13}C NMR ($CDCl_3$, 70 MHz): δ 55.3, 55.4, 114.1, 114.8, 116.3, 120.3, 122.0, 124.5, 127.1, 129.5, 129.9, 130.1, 138.0, 144.8, 150.9, 153.8, 158.1, 160.1; EIMS, *m*/*z*: 419 $(M + 2)^+$, 417 (M^+); Anal. Calcd. For $C_{24}H_{20}ClN_3O_2$ (417.88): C, 68.98; H, 4.82; N, 10.06. Found: C, 69.12; H, 4.89; N, 10.38.

*4.1.3.6. 4-Chloro-N-((1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)methylene)aniline (**5f**).* Yield 52%; m.p. 186–188 °C; IR (KBr, cm^{-1}): 3012 (CH aromatic), 2920, 2850 (CH aliphatic); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 3H, OCH_3), 7.01–7.90 (m, 13H, aromatic H+ CH of pyrazole), 8.49 (s, 1H, HC=N); EIMS, *m*/*z*: 421 (M^+); Anal. Calcd. For $C_{23}H_{17}Cl_2N_3O$ (422.30): C, 65.41; H, 4.06; N, 9.95. Found: C, 65.38; H, 4.09; N, 10.08.

*4.1.3.7. 4-(4-((Phenylimino)methyl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**5g**).* Yield 67%; m.p. 152–154 °C; IR (KBr, cm^{-1}): 3321, 3251 (NH_2), 3062, 3024 (CH aromatic), 2916 (CH aliphatic), 1342, 1157 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.43 (s, 3H, CH_3), 6.68–8.03 (m, 13H, aromatic H), 8.06 (s, ex, 2H, NH_2), 8.50 (s, 1H, CH of pyrazole), 8.60 (s, 1H, HC=N); EIMS, *m*/*z*: 416 (M^+); Anal. Calcd. For $C_{23}H_{20}N_4O_2S$ (416.49): C, 66.33; H, 4.84; N, 13.45. Found: C, 66.52; H, 4.96; N, 13.80.

*4.1.3.8. 4-(4-((4-Methoxyphenylimino)methyl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**5h**).* Yield 62%; m.p. 130–132 °C; IR (KBr, cm^{-1}): 3379, 3298 (NH_2), 3062 (CH aromatic), 2920, 2835 (CH aliphatic), 1338, 1161 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.43 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.67–8.03 (m, 12H, aromatic H), 8.46 (s, 1H, CH of pyrazole), 8.59 (s, 1H, HC=N), 9.16 (s, ex, 2H, NH_2); EIMS, *m/z*: 446 (M^+); Anal. Calcd. For $C_{24}H_{22}N_4O_3S$ (446.52): C, 64.56; H, 4.97; N, 12.55. Found: C, 64.79; H, 5.31; N, 12.70.

*4.1.3.9. 4-(4-((4-Chlorophenylimino)methyl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**5i**).* Yield 61%; m.p. 138–140 °C; IR (KBr, cm^{-1}): 3360, 3250(NH_2), 3012 (CH aromatic), 2924 (CH aliphatic), 1342, 1149 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 2.38 (s, 3H, CH_3), 7.08–8.02 (m, 12H, aromatic H), 8.47 (s, 1H, CH of pyrazole), 8.68 (s, 1H, HC=N), 8.80 (s, ex, 2H, NH_2); EIMS, *m/z*: 450 (M^+); Anal. Calcd. For $C_{23}H_{19}ClN_4O_2S$ (450.94): C, 61.26; H, 4.25; N, 12.42. Found: C, 61.56; H, 4.50; N, 12.08.

*4.1.3.10. 4-(3-(4-Methoxyphenyl)-4-((phenylimino)methyl)-1H-pyrazol-1-yl)benzenesulfonamide (**5j**).* Yield 66%; m.p. 113–115 °C; IR (KBr, cm^{-1}): 3321, 3251 (NH_2), 3012 (CH aromatic), 2924, 2854 (CH aliphatic), 1342, 1157 (SO_2); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.89 (s, 3H, OCH_3), 7.02–8.05 (m, 13H, aromatic H), 8.52 (s, 1H, CH of pyrazole), 8.62 (s, 1H, HC=N), 8.70 (s, ex, 2H, NH_2); EIMS *m/z*: 432 (M^+); Anal. Calcd. For $C_{23}H_{20}N_4O_3S$ (432.49): C, 63.87; H, 4.66; N, 12.95. Found: C, 63.94; H, 4.71; N, 13.14.

*4.1.3.11. 4-(3-(4-Methoxyphenyl)-4-((4-methoxyphenylimino) methyl)-1H-pyrazol-1-yl)benzenesulfonamide (**5k**).* Yield 61%; m.p. 124–126 °C; IR (KBr, cm^{-1}): 3379, 3250 (NH_2), 3066 (CH aromatic), 2931, 2835 (CH aliphatic), 1342, 1145 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 3.82 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.93–8.02 (m, 12H, aromatic H), 8.06 (s, ex, 2H, NH_2), 8.46 (s, 1H, CH of pyrazole), 8.57 (s, 1H, HC=N); EIMS, *m/z*: 462 (M^+); Anal. Calcd. For $C_{24}H_{22}N_4O_4S$ (462.52): C, 62.32; H, 4.79; N, 12.11. Found: C, 62.42; H, 4.72; N, 12.38.

*4.1.3.12. 4-(4-((4-Chlorophenylimino)methyl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**5l**).* Yield 60%; m.p. 118–119 °C; IR (KBr, cm^{-1}): 3360, 3244 (NH_2), 3074 (CH aromatic), 2931, 2835 (CH aliphatic), 1342, 1149 (SO_2); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 3H, OCH_3), 6.60–8.05 (m, 12H, aromatic H), 8.47 (s, 1H, CH of pyrazole), 8.62 (s, 1H, HC=N), 8.80 (s, ex, 2H, NH_2); EIMS, *m/z*: 466 (M^+); Anal. Calcd. For $C_{23}H_{19}ClN_4O_3S$ (466.93): C, 59.16; H, 4.10; N, 12.00. Found: C, 59.39; H, 4.22; N, 12.31.

*4.1.4. General procedure for the synthesis of compounds (**6a**–**d**)*

To a mixture of **3a**,**b** or **4a**,**b** (10 mmol) and 4-chloroacetophenone (1.54 g, 10 mmol) in absolute ethanol (20 mL), sodium hydroxide (1.20 g, 30 mmol) in water (3 mL) was added. The reaction mixture was stirred at room temperature for 24 h. Then, diluted with water and acidified with 10% hydrochloric acid till pH = 5. The precipitated solid was filtered and crystallized from methanol.

*4.1.4.1. 1-(4-Chlorophenyl)-3-(1-(4-chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)prop-2-en-1-one (**6a**).* Yield 79%; m.p. 205–206 °C; IR (KBr, cm^{-1}): 3066 (CH aromatic), 2916 (CH aliphatic), 1662 (C=O); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 7.57 (d, 1H, CH olefinic, J = 8.4 Hz), 7.74 (d, 1H, CH olefinic, J = 9.0 Hz), 7.29–7.49, 7.86–7.92 (m, 12H, aromatic H), 8.32 (s, 1H, CH pyrazole); EIMS, *m/z*: 436 (M + 4)$^+$, 434 (M + 2)$^+$, 432 (M^+); Anal. Calcd. For $C_{25}H_{18}Cl_2N_2O$ (433.32): C, 69.29; H, 4.19; N, 6.46, Found: C, 69.38; H, 4.23; N, 6.69.

*4.1.4.2. 1-(4-Chlorophenyl)-3-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)prop-2en-1-one (**6b**).* Yield 89%; m.p. 201–202 °C; IR (KBr, cm^{-1}): 3066, 3005 (CH aromatic), 2958, 2935 (CH aliphatic), 1662 (C=O); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 3H, OCH_3), 7.01 (d, 1H, CH olefinic, J = 8.4 Hz), 7.60 (d, 1H, CH olefinic, J = 8.4 Hz) 7.26–7.46, 7.72–7.91 (m, 12H, aromatic H), 8.29 (s, 1H, CH pyrazole); ^{13}C NMR ($CDCl_3$, 70 MHz): δ 55.3, 114.2, 118.3, 120.3, 120.9, 124.4, 126.6, 128.8, 129.6, 129.7, 129.9, 132.6, 135.7, 136.4, 137.8, 145.0, 153.9, 160.2, 188.5; EIMS, *m/z*: 452 (M + 4)$^+$, 450 (M + 2)$^+$, 448 (M^+); Anal. Calcd. For $C_{25}H_{18}Cl_2N_2O_2$ (449.32): C, 66.83; H, 4.04; N, 6.23. Found: C, 66.91; H, 4.08; N, 6.38.

*4.1.4.3. 4-(4-(3-(4-Chlorophenyl)-3-oxoprop-1-enyl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**6c**).* Yield 85%; m.p. 105–107 °C; IR (KBr, cm^{-1}): 3387, 3263 (NH_2), 3086, 3012 (CH aromatic), 2958, 2927 (CH aliphatic), 1678 (C=O), 1384, 1161 (SO_2); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, CH_3), 7.43 (d, 1H, CH olefinic, J = 8.7 Hz), 7.89 (d, 1H, CH olefinic, J = 8.1 Hz), 7.17–7.38, 7.80–7.82 (m, 14H, aromatic H+ ex, NH_2), 8.02 (s, 1H, CH pyrazole); EIMS, *m/z*: 477 (M^+); Anal. Calcd. For $C_{25}H_{20}ClN_3O_3S$ (477.96): C, 62.82; H, 4.22; N, 8.79. Found: C, 63.25; H, 4.43; N, 8.82.

*4.1.4.4. 4-(4-(3-(4-Chlorophenyl)-3-oxoprop-1-enyl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**6d**).* Yield 87%; m.p. 171–173 °C; IR (KBr, cm^{-1}): 3325, 3244 (NH_2), 3124 (CH aromatic), 2958, 2927 (CH aliphatic), 1670 (C=O), 1330, 1165 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 3.82 (s, 3H, OCH_3), 6.90–7.87 (m, 16H, aromatic H+ olefinic H+ ex, NH_2), 7.98 (s, 1H, CH pyrazole); EIMS, *m/z*: 492 (M − 1)$^+$; Anal. Calcd. For $C_{25}H_{20}ClN_3O_4S$ (493.96): C, 60.79; H, 4.08; N, 8.51. Found: C, 61.07; H, 4.21; N, 8.79.

*4.1.5. General procedure for the synthesis of compounds (**7a**–**h**)*

Compounds **6a**–**d** (3 mmol) were dissolved in the minimum amount of DMF, then 4-substituted benzaldehyde (3 mmol) was added to the solutions followed by KCN (0.30 mmol). The reaction mixture was stirred at (30–35 °C) for 4 h, then poured onto ice water and neutralized with drops of concentrated hydrochloric acid; the precipitated solid was filtered washed with water and crystallized from methanol.

*4.1.5.1. 4-(4-Chlorophenyl)-2-(1-(4-chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)-1-(4-fluorophenyl)butane-1,4-dione (**7a**).* Yield 78%; m.p. 143–145 °C; IR (KBr, cm^{-1}): 3040 (CH aromatic), 2920 (CH aliphatic), 1681 (C=O); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.40 (s, 3H, CH_3), 3.26 (dd, 1H, CH–C=O, J = 17.1, 5.1 Hz), 3.45 (dd, 1H, CH–C=O, J = 17.1, 8.0 Hz), 4.26 (dd, 1H, CH, J = 8.0, 5.1 Hz), 7.20–7.83 (m, 16H, aromatic H), 7.88 (s, 1H, CH pyrazole); EIMS, *m/z*: 557 (M + 1)$^+$; Anal. Calcd. For $C_{32}H_{23}Cl_2FN_2O_2$ (557.44): C, 68.95; H, 4.16; N, 5.03. Found: C, 69.02; H, 4.14; N, 5.17.

*4.1.5.2. 4-(4-Chlorophenyl)-2-(1-(4-chlorophenyl)-3-p-tolyl-1H-pyrazol-4-yl)-1-(4-methoxyphenyl)butan-1,4-dione (**7b**).* Yield 52%; m.p. 114–116 °C; IR (KBr, cm^{-1}): 3070 (CH aromatic), 2920, 2839 (CH aliphatic), 1678 (C=O); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.44 (s, 3H, CH_3), 3.25 (dd, 1H, CH–C=O, J = 16.8, 6.0 Hz), 3.45 (dd, 1H, CH–C=O, J = 16.8, 8.7 Hz), 3.87 (s, 3H, OCH_3), 4.23 (dd, 1H, CH, J = 8.7, 6.0 Hz), 7.00–7.84 (m, 16H, aromatic H), 7.87 (s, 1H, CH pyrazole); EIMS, *m/z*: 568 (M^+); Anal. Calcd. For $C_{33}H_{26}Cl_2N_2O_3$ (569.47): C, 69.60; H, 4.60; N, 4.92. Found: C, 69.65; H, 4.58; N, 5.12.

*4.1.5.3. 4-(4-Chlorophenyl)-2-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1-(4-fluorophenyl)butan-1,4-dione (**7c**).* Yield 65%; m.p. 170–171 °C; IR (KBr, cm^{-1}): 3070, 3000 (CH aromatic), 2931, 2916 (CH aliphatic), 1681 (C=O); ^1H NMR ($CDCl_3$,

300 MHz): δ 3.38 (dd, 1H, CH–C═O, J = 17.7, 5.1 Hz), 3.59 (dd, 1H, CH–C═O, J = 17.7, 8.4 Hz), 3.89 (s, 3H, OCH_3), 4.70 (dd, 1H, CH, J = 8.4, 5.1 Hz), 6.99–7.80 (m, 16H, aromatic H), 8.11 (s, 1H, CH pyrazole); EIMS, *m/z*: 572 (M^+); Anal. Calcd. For $C_{32}H_{23}Cl_2FN_2O_3$ (573.44): C, 67.02; H, 4.04; Cl, 12.37; N, 4.89. Found: C, 67.05; H, 4.10; Cl, 12.46; N, 4.98.

*4.1.5.4. 4-(4-Chlorophenyl)-2-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1-(4-methoxyphenyl)butane-1,4-dione (**7d**).* Yield 46%; m.p. 108–110 °C; IR (KBr, cm^{-1}): 3070 (CH aromatic), 2924, 2854 (CH aliphatic), 1678 (C═O); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.23 (dd, 1H, CH–C═O, J = 16.7, 6.0 Hz), 3.43 (dd, 1H, CH–C═O, J = 16.7, 8.5 Hz), 3.78–3.98 (m, 6H, $2OCH_3$), 4.23 (dd, 1H, CH, J = 8.5, 6.0 Hz), 6.88–7.82 (m, 16H, aromatic H), 8.10 (s, 1H, CH pyrazole); ^{13}C NMR ($CDCl_3$, 70 MHz): δ 44.3, 50.8, 55.2, 55.5, 114.0, 114.1, 114.2, 114.3, 118.6, 119.8, 119.8, 123.5, 125.5, 128.2, 128.3, 128.5, 128.6, 129.1, 129.2, 129.3, 129.4, 129.5, 130.1, 130.7, 131.0, 134.8, 137.5, 138.2, 151.5, 159.5, 160.3, 197.4; EIMS, *m/z*: 585 $(M + 1)^+$; Anal. Calcd. For $C_{33}H_{26}Cl_2N_2O_4$ (585.47): C, 67.70; H, 4.48; N, 4.78. Found: C, 67.74; H, 4.53; N, 4.89.

*4.1.5.5. 4-(4-(4-Chlorophenyl)-1-(4-fluorophenyl)-1,4-dioxobutan-2-yl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**7e**).* Yield 87%; m.p. 112–114 °C; IR (KBr, cm^{-1}): 3340, 3248 (NH_2), 3070 (CH aromatic), 2924, 2858 (CH aliphatic), 1666 (C═O), 1161, 1338 (SO_2); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.35 (s, 3H, CH_3), 3.22 (dd, 1H, CH–C═O, J = 16.8, 5.1 Hz), 3.46 (dd, 1H, CH–C═O, J = 16.8, 8.4 Hz), 4.24 (dd, 1H, CH, J = 8.4, 5.1 Hz), 6.85–7.99 (m, 16H, aromatic H), 8.37 (s, ex, 2H, NH_2), 8.55 (s, 1H, CH pyrazole); EIMS, *m/z*: 603 $(M + 1)^+$; Anal. Calcd. For $C_{32}H_{25}ClFN_3O_4S$ (602.07): C, 63.84; H, 4.19; N, 6.98. Found: C, 63.93; H, 4.25; N, 7.17.

*4.1.5.6. 4-(4-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1,4-dioxobutan-2-yl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**7f**).* Yield 91%; m.p. 183–186 °C; IR (KBr, cm^{-1}): 3352, 3263 (NH_2), 3070 (CH aromatic), 2924 (CH aliphatic), 1674 (C═O), 1338, 1161 (SO_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.44(s, 3H, CH_3), 3.25 (dd, 1H, CH–C═O, J = 17.1, 5.1 Hz), 3.46 (dd, 1H, CH–C═O, J = 17.1, 7.9 Hz), 3.90 (s, 3H, OCH_3), 4.23 (dd, 1H, CH, J = 7.9, 5.1 Hz), 6.89–7.91 (m, 16H, aromatic H), 8.03 (s, 1H, CH pyrazole), 8.37 (s, ex, 2H, NH_2); EIMS, *m/z*: 616 $(M + 2)^+$, 614 (M^+); Anal. Calcd. For $C_{33}H_{28}ClN_3O_5S$ (614.11): C, 64.54; H, 4.60; N, 6.84. Found: C, 64.48; H, 4.63; N, 6.98.

*4.1.5.7. 4-(4-(4-Chlorophenyl)-1-(4-fluorophenyl)-1,4-dioxobutan-2-yl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**7g**).* Yield 95%; m.p. 123–125 °C; IR (KBr, cm^{-1}): 3340, 3240 (NH_2), 3070 (CH aromatic), 2924, 2858 (CH aliphatic), 1678 (C═O), 1338, 1161 (SO_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.45 (dd, 1H, CH–C═O, J = 17.1, 5.4 Hz), 3.61 (dd, 1H, CH–C═O, J = 17.1, 8.0 Hz), 3.88 (s, 3H, OCH_3), 4.19 (dd, 1H, CH, J = 8.0, 5.4 Hz), 6.88–7.91, (m, 16H, aromatic H), 8.69 (s, 1H, CH pyrazole), 9.38 (s, ex, 2H, NH_2); EIMS, *m/z*: 620 $(M + 2)^+$, 618 (M^+); Anal. Calcd. For $C_{32}H_{25}ClFN_3O_5S$ (618.07): C, 62.18; H, 4.08; N, 6.80. Found: C, 62.22; H, 4.15; N, 6.97.

*4.1.5.8. 4-(4-(4-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1,4-dioxobutan-2-yl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**7h**).* Yield 50%; m.p. 182–185 °C; IR (KBr, cm^{-1}): 3321, 3244 (NH_2), 3124, 3074 (CH aromatic H), 2924, 2835 (CH aliphatic), 1670 (C═O), 1330, 1165 (SO_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.26 (dd, 1H, CH–C═O, J = 16.7, 6.0 Hz), 3.46 (dd, 1H, CH–C═O, J = 16.7, 8.4 Hz), 3.71–3.90 (m, 6H, $2OCH_3$), 4.23 (dd, 1H, CH–C═O, J = 8.4, 6.0 Hz), 6.90–7.86 (m, 16H, aromatic H), 8.03 (s, 1H, CH pyrazole), 8.30 (s, ex, 2H, NH_2); EIMS, *m/z*: 629 $(M - 1)^+$; Anal. Calcd. For $C_{33}H_{28}ClN_3O_6S$ (630.10): C, 62.90; H, 4.48; N, 6.67. Found: C, 62.97; H, 4.53; N, 6.81.

*4.1.6. General procedure for the synthesis of compounds (**8a**–**h**)*

Compounds **7a**–**h** (2 mmol) was added to stirred solution of ammonium acetate (2 mmol) in glacial acetic acid (5 mL). The reaction mixture was refluxed at 110 °C for 20 h, poured onto ice water. The precipitate formed was filtered washed with water, dried and crystallized from ethanol.

*4.1.6.1. 1-(4-Chlorophenyl)-4-(5-(4-chlorophenyl)-2-(4-fluorophenyl)-1H-pyrrol-3-yl)-3-p-tolyl-1H-pyrazole (**8a**).* Yield 74%; m.p. 132–134 °C; IR (KBr, cm^{-1}): 3417 (NH), 3059 (CH aromatic), 2916, 2850 (CH aliphatic); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.43 (s, 3H, CH_3), 5.00 (s, ex, 1H, NH), 6.97 (s, 1H, CH of pyrrole), 6.98–8.10 (m, 16H, aromatic H), 8.25 (s, 1H, CH of pyrazole); EIMS, *m/z*: 541 $(M + 4)^+$, 539 $(M + 2)^+$, 537 (M^+); Anal. Calcd. For $C_{32}H_{22}Cl_2FN_3$ (538.44): C, 71.38; H, 4.12; N, 7.80. Found: C, 71.41; H, 4.15; N, 7.94.

*4.1.6.2. 1-(4-Chlorophenyl)-4-(5-(4-chlorophenyl)-2-(4-methoxyphenyl)-1H-pyrrol-3-yl)-3-p-tolyl-1H-pyrazole (**8b**).* Yield 78%; m.p. 135–137 °C; IR (KBr, cm^{-1}): 3390 (NH), 3062, 3020 (CH aromatic), 2920, 2850 (CH aliphatic); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.43 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 5.05 (s, ex, 1H, NH), 6.65 (s, 1H, CH of pyrrole), 6.98–7.95 (m, 16H, aromatic H), 8.25 (s, 1H, CH pyrazole); EIMS, *m/z*: 550 $(M + 1)^+$; Anal. Calcd. For $C_{33}H_{25}Cl_2N_3O$ (550.47): C, 72.00; H, 4.58; N, 7.63. Found: C, 71.98; H, 4.59; N, 7.76.

*4.1.6.3. 1-(4-Chlorophenyl)-4-(5-(4-chlorophenyl)-2-(4-fluorophenyl)-1H-pyrrol-3-yl)-3-(4-methoxyphenyl)-1H-pyrazole (**8c**).* Yield 76%; m.p. 120–122 °C; IR (KBr, cm^{-1}): 3390 (NH), 3070 (CH aromatic), 2920, 2850 (CH aromatic); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 3H, OCH_3), 5.30 (s, ex, 1H, NH), 6.50 (s, 1H, CH of pyrrole), 7.02–8.15 (m, 16H, aromatic H), 8.25 (s, 1H, CH pyrazole); EIMS, *m/z*: 557 $(M + 4)^+$, 555 $(M + 2)^+$, 553 (M^+); Anal. Calcd. For $C_{32}H_{22}Cl_2FN_3O$ (554.44): C, 69.32; H, 4.00; N, 7.58. Found: C, 69.36; H, 4.05; N, 7.69.

*4.1.6.4. 1-(4-Chlorophenyl)-4-(5-(4-chlorophenyl)-2-(4-methoxyphenyl)-1H-pyrrol-3-yl)-3-(4-methoxyphenyl)-1H-pyrazole (**8d**).* Yield 84%; m.p. 155–157 °C; IR (KBr, cm^{-1}): 3390 (NH), 3066 (CH aromatic), 3920, 2850 (CH aliphatic); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.73–3.88 (m, 6H, $2OCH_3$), 5.05 (s, ex, 1H, NH), 6.55 (s, 1H, CH of pyrrole), 7.01–8.15 (m, 16H, aromatic H), 8.35 (s, 1H, CH pyrazole); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 55.2, 110.0, 113.0, 114.1, 120.0, 120.2, 128.1, 128.8, 129.1, 129.3, 129.5, 129.9, 131.3, 130.2, 137.5, 138.0, 160.0; EIMS, *m/z*: 566 $(M + 1)^+$; Anal. Calcd. For $C_{33}H_{25}Cl_2N_3O_2$ (566.47): C, 69.97; H, 4.45; N, 7.42. Found: C, 70.02; H, 4.47; N, 7.58.

*4.1.6.5. 4-(4-(5-(4-Chlorophenyl)-2-(4-fluorophenyl)-1H-pyrrol-3-yl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**8e**).* Yield 78%; m.p. 185–188 °C; IR (KBr, cm^{-1}): 3402, 3244 (NH_2, NH), 3066, 3032 (CH aromatic H), 2920, 2850 (CH aliphatic), 1338, 1157 (SO_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.29 (s, 3H, CH_3), 7.05 (s, 1H, CH of pyrrole), 5.90 (s, ex, 1H, NH), 7.16 (s, ex, 2H, NH_2), 7.27–8.14 (m, 16H, aromatic), 9.02 (s, 1H, CH pyrazole); EIMS, *m/z*: 582 $(M - 1)^+$; Anal. Calcd. For $C_{32}H_{24}ClFN_4O_2S$ (583.07): C, 65.92; H, 4.15; N, 9.61. Found: C, 65.92; H, 4.13; N, 9.83.

*4.1.6.6. 4-(4-(5-(4-Chlorophenyl)-2-(4-methoxyphenyl)-1H-pyrrol-3-yl)-3-p-tolyl-1H-pyrazol-1-yl)benzenesulfonamide (**8f**).* Yield 85%; m.p. 204–205 °C; IR (KBr, cm^{-1}): 3383, 3255 (NH_2, NH), 3070, 3032 (CH aromatic), 2920, 2850 (CH aliphatic), 1338, 1161 (SO_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.44 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.85 (s, ex, 1H, NH), 6.85 (s, 1H, CH of pyrrole), 7.13–8.05 (m, 16H, aromatic H), 8.14 (s, 1H, CH pyrazole), 9.32 (s, ex, 2H, NH_2); EIMS, *m/z*: 597 $(M + 2)^+$, 595 (M^+); Anal. Calcd. For $C_{33}H_{27}ClN_4O_3S$ (595.11): C, 66.60; H, 4.57; N, 9.41. Found: C, 66.71; H, 4.52; N, 9.54.

*4.1.6.7. 4-(4-(5-(4-Chlorophenyl)-2-(4-fluorophenyl)-1H-pyrrol-3-yl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**8g**).* Yield 58%; m.p. 188–190 °C; IR (KBr, cm^{-1}): 3402, 3240 (NH_2, NH), 3070, 3032 (CH aromatic), 2924, 2850 (CH aliphatic), 1338, 1161 (SO_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 3.71 (s, 3H, OCH_3), 5.24 (s, ex, 1H, NH), 6.93 (s, 1H, CH of pyrrole), 6.94–7.97 (m, 18H, aromatic H+ ex, NH_2), 8.30 (s, 1H, CH pyrazole); EIMS, *m/z*: 599 (M^+); Anal. Calcd. For $C_{32}H_{24}ClFN_4O_3S$ (599.07): C, 64.16; H, 4.04; N, 9.35. Found: C, 64.21; H, 4.09; N, 9.51.

*4.1.6.8. 4-(4-(5-(4-Chlorophenyl)-2-(4-methoxyphenyl)-1H-pyrrol-3-yl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide (**8h**).* Yield 55%; m.p. 197–200 °C; IR (KBr, cm^{-1}): 3387, 3248 (NH_2, NH), 3109, 3078 (CH aromatic), 2920, 2846 (CH aliphatic), 1338, 1161 (SO_2); ^1H NMR (DMSO-d_6, 500 MHz): δ 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 5.11 (s, ex, 1H, NH), 6.88 (s, 1H, CH of pyrrole), 7.00–8.15 (m, 16H, aromatic H), 8.70 (s, 1H, CH pyrazole), 9.22 (s, ex, 2H, NH_2); EIMS, *m/z*: 613 $(M + 2)^+$, 611 (M^+); Anal. Calcd. For $C_{33}H_{27}ClN_4O_4S$ (611.10): C, 64.86; H, 4.45; N, 9.17. Found: C, 64.91; H, 4.42; N, 9.32.

4.2. Pharmacological screening

4.2.1. Anti-inflammatory (in vivo screening)

All the target compounds were evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema method of Winter et al. [35]. The employed technique is based on the ability of the tested compounds to inhibit the edema produced in the hind paw of the rat after injection of carrageenan. Male Wister albino rats (obtained from the animal house of Faculty of Pharmacy, Cairo University) weighing 120–180 g were used to investigate the carrageenan-induced rat paw edema. The rats were kept in animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into 38 groups of five rats each. The initial hind paw volume of rats was determined volumetrically by means of plethysmometer 7150 (UGO Basile, Italy). Phenylbutazone (reference standard) and the tested compounds suspended in 2% Tween 80 were administered intraperitoneally at a dose of 0.32 mmol/kg body weight, while the control group received only 2% Tween 80, 1 h before induction of inflammation. The paw edema was induced by sub-plantar injection of (0.1 mL) of 1% carrageenan solution in saline (0.9%). Paw edema volume was measured after 2 and 3 h using the plethysmometer and compared with the initial hind paw volume of each rat. The difference of average values between treated and control group is calculated for each time interval and evaluated statistically. Quantitative variables from normal distribution were expressed as means ± standard error (SEM). The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group according to the following equation:

$$\%\ \text{Inhibition} = (\text{Vc} - \text{Vt})100/\text{Vc}$$

where Vc is the mean of edema volume of rat paw after administration of carrageenan in the control group, Vt is the mean of edema volume of rat paw after administration of the tested compounds or the reference drugs.

The IC_{50} values of the most active compounds **5e** and **8e** and phenylbutazone were applied according to the previous reported procedure in doses of 0.08, 0.16, and 0.32 mmol/kg body weight.

4.2.2. Analgesic activity

Compounds that exhibited good anti-inflammatory activity and phenylbutazone were screened for their analgesic activity using the reported method of *p*-benzoquinone-induced writhing in mice by Okun et al. [36] Adult male albino mice weighing 20–25 g were used in this study. Phenylbutazone (reference standard) and the tested compounds were prepared as suspension in 2% Tween 80. A sensitivity test was carried out one day before drug administration, were the animals were injected intraperitoneally with 0.2–0.25 mL of 0.02% freshly prepared solution of *p*-benzoquinone in distilled water. Animals showing writhing to *p*-benzoquinone within 30 min were chosen for studying the analgesic activity. On the next day, mice were divided into 19 groups, 6 animals each. The control group received only 2% Tween 80 while the rest of the groups received phenylbutazone and the tested compounds at a dose of 0.32 mmol/kg body weight. After 1 h, 0.02% solution of *p*-benzoquinone was administered intraperitoneally and the animals were observed for 30 min after injection of the irritant, the animals showing writhing were counted in each group. Writhing is known as stretch, torsion to one side, drawing up of hind leg, retraction of the abdomen, so that the belly of mouse touches the floor. The mice showing any of the previous signs are counted as positive responses and this method depend on the ability of tested compounds to protect the animals from writhing signs made by *p*-benzoquinone. The analgesic activity was evaluated as the percentage protection of tested animals against irritant *p*-benzoquinone induced writhing response compared with the control group according the following equation:

$$\%\ \text{protection} = \text{Number of protected animals} \times 100/\text{total number of animals}$$

4.2.3. Ulcerogenic effect

The ulcerogenic effect of the nine active compounds as anti-inflammatory agents and phenylbutazone was evaluated by the reported method of Meshali et al. [37] Adult male albino rats weighing 120–180 g were used in this study. Animals were fasted 18 h before the drug administration, then divided into 11 groups each of 5 animals and received the drug orally. The first group received 2% Tween 80 and kept as control, the second group received phenylbutazone in a dose of 0.32 mmol/kg body weight and the rest of the groups were received the tested compounds in the same dose.

Food was allowed 2 h after administration of the drugs and rats were received the same dose orally for three successive days. Two hours after the last dose, rats were sacrificed, the stomach of each rat were removed, opened along greater curvature and cleaned by washing with cold saline. The stomach was stretched on a cork-board using pins and examined with a magnifying lens (10×) for the presence of ulcers and erosions. Ulcer index was calculated according to the method of Robert et al. [38]. The degree of ulcerogenic effect was expressed in term of; percentage incidence of ulcer in each group of animals divided by 10, the average number of ulcers per stomach and the average severity of ulcers by visual observation. The ulcer index was expressed as summation value of the above three values.

4.2.4. Acute toxicity

LD_{50} of some representative compounds was determined using Finney's method [39]. Adult male mice weighing 20–25 g were divided into groups each of six animals. Minimal dose that killed all animals and the maximal dose that failed to kill any animal were determined via several increasing intraperitoneal doses. Animals were kept under observation for 24 h during which any mortality in each group was recorded.

4.2.5. In vitro COX inhibition study

The most active compounds as anti-inflammatory **5e**,**f**, **8e**, and indomethacin were tested for their ability to inhibit COX-1 and/or

COX-2 using Cayman's COX-Activity Assay kit (catalog no. 760151, Cayman Chemicals, Ann Arbor, MI, USA) which measure the peroxidase activity of COX by the method of Kulmacz and Lands [40]. COX-1/COX-2 percentage inhibition activity ratio of the tested compounds and indomethacin was calculated and recorded in Table 5.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.005.

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