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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of some pyrazole derivatives of expected anti-inflammatory and analgesic activities

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Abstract Design and synthesis of some pyrazole derivatives **4–11** of expected anti-inflammatory and analgesic activities. In addition, docking of the tested compounds into cyclooxygenase II using (MOE) was performed to rationalize the obtained biological results and their mechanism of action. The structures of the new compounds were elucidated by spectral and elemental analyses. All the newly synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan-induced rat paw edema method. Analgesic activity of the target compounds was measured using the *p*-benzoquinone writhing-induced method, and their ability to induce gastric toxicity was also evaluated. Results showed that the newly synthesized compounds exhibited weak to good activities compared to ibuprofen and celecoxib as reference drugs. Some compounds, such **4a** and **11b** exhibited significant anti-inflammatory activity with gastric ulcerogenic potential less than that of ibuprofen. Results of the analgesic activity showed that compounds possessing good anti-inflammatory activity showed also good analgesic. Substitution of pyrazole ring with at least one aryl moiety was found to be essential for anti-inflammatory and analgesic activities. Free NH (of pyrazole ring) and/or acidic group (COOH) will improve the anti-inflammatory activity.

Keywords Pyrazoles · Anti-inflammatory · Analgesic · MOE

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Introduction

Inflammation is a normal and essential response to any noxious stimulus, which threatens the host and may vary from a localized response to a more generalized one (Fylaktakidou *et al.*, 2004). The inflammatory process is designed to provide a rapid mechanism by which the host can respond to the invasion of foreign materials and return to homeostatic equilibrium. Acute inflammation is mediated by the release of autacoids such as histamine, serotonin, bradykinin, prostaglandins, and leukotrienes (Lacerda *et al.*, 2009). On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon, and tumor necrosis factor α (TNF-α), a cytokine that plays a major role in this kind of inflammatory process and whose production is associated with some inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and others (Lacerda *et al.*, 2009).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most useful clinical therapies for the treatment of pain, fever, and inflammation (Laufer and Luik, 2010). The major mechanism by which NSAIDs exert their anti-inflammatory activity is by the inhibition of cyclooxygenase-derived prostaglandin synthesis, which is also responsible for the gastrointestinal (Sostres *et al.*, 2010; Naesdal and Brown, 2006; Cryer, 2005; Lazzaroni and Bianchi Porro, 2004; James and Hawkey, 2003), renal (Schneider *et al.*, 2006; Mounier *et al.*, 2006; Zadrazil, 2006), and hepatic (Yanai *et al.*, 2009) side effects that are observed mainly in chronic use of NSAIDs. Therefore, the challenge still exists for the pharmaceutical industry to develop effective anti-inflammatory agents with enhanced safety profile. Literature survey revealed that many 2-pyrazoline derivatives have been reported to exhibit various

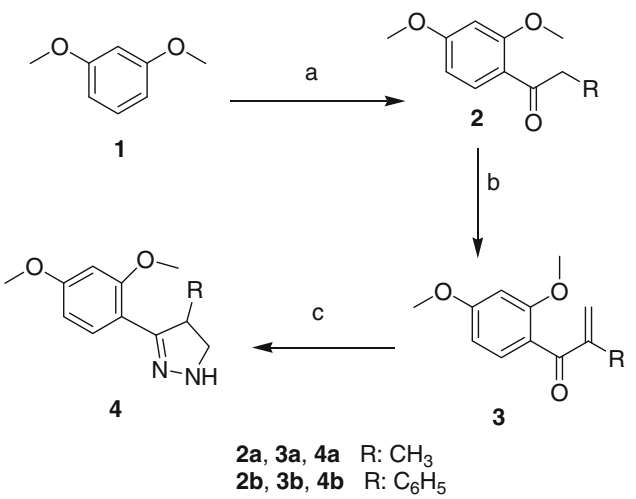
pharmacological activities such as antimicrobial (Karthikeyan *et al.*, 2007; Ramiz *et al.*, 2010), anti-inflammatory (Barsoum *et al.*, 2006; Shoman *et al.*, 2009; Rathish *et al.*, 2009; Abbas *et al.*, 2010), and antihypertensive (Turan-Zitouni *et al.*, 2000). Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib is the one which is treated as a safe anti-inflammatory and analgesic agent. Some examples of pyrazole derivatives as NSAIDs are mefobutazone, morazone, famprofazone, and ramifenazone (Reynold, 1993; Menozzi *et al.*, 2003; Sakya *et al.*, 2007; Patel *et al.*, 2004).

Motivated by the aforementioned findings, it was designed to synthesize novel series of pyrazole derivatives that would act as anti-inflammatory agents with reduced gastric toxicity. The synthesized compounds were tested in vivo for their anti-inflammatory and analgesic activities as well as their ulcerogenic liability. In order to rationalize the pharmacological results obtained and the mechanism of action, MOE (Molecular Operating Environment, 2005) docking studies of the synthesized compounds were performed using murine cyclooxygenase-2 co-crystallized with celecoxib (Protein Data Bank code PDB ID: 6COX) as a template.

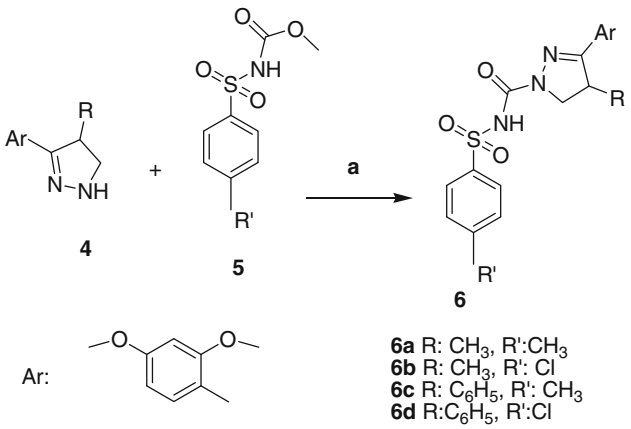
Result and discussion

Chemistry

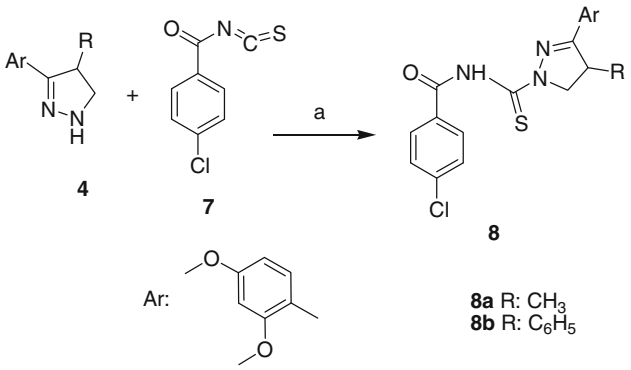
The synthesis routes for the preparation of the target compounds were illustrated in Schemes 1, 2, 3, and 4. Synthesis of the appropriate 1-(2,4-dimethoxyphenyl)alkyl/aryl ketones **2a** and **2b** was carried out via the reaction of 1,3-dimethoxybenzene with propionic acid or phenyl acetic



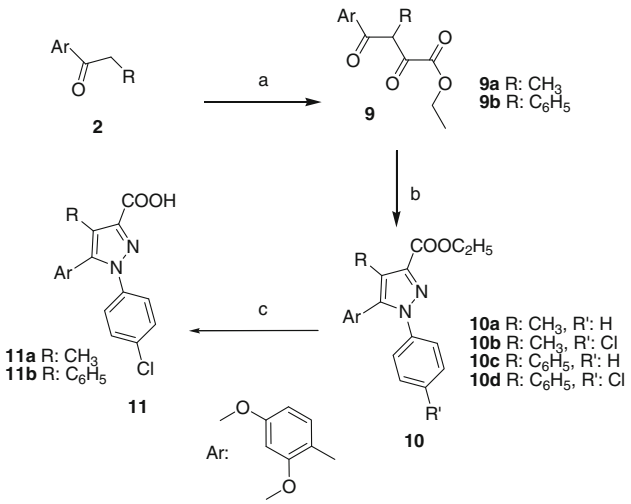
Scheme 1 Reagents and solvents: **a** RCH_2COOH/PAA, **b** HCHO/piperdine/acetic acid, and **c** $NH_2NH_2{\cdot}H_2O$/ethanol



Scheme 2 Reagents and solvents: **a** toluene



Scheme 3 Reagents and solvents: **a** CH_3CN, 0°C



Scheme 4 Reagents and solvents: **a** $(COOC_2H_5)_2$/NaH/toluene, **b** $R'C_6H_4NHNH_2{\cdot}HCl$/triethylamine/ethanol, and **c** KOH/methanol

acid in polyphosphoric acid (Slotta and Heller, 1930). The structure of **2b** was confirmed by appearance of a singlet at δ 4.29 ppm corresponding to methylene protons.

Compounds **3a** and **3b** were synthesized from **2a** and **2b**, respectively, using Mannich reaction/elimination sequence. ^{1}H-NMR spectrum of **3a** had two doublets due to geminal coupling at δ 2.93 (J: 7.4 Hz) and 3.04 ppm (J: 7.4 Hz) corresponding to the methylene protons and that of **3b** appeared at δ 4.21 (J: 8.2 Hz) and 4.86 ppm (J: 8.2 Hz). Moreover, cyclocondensation of **3a** and **3b** with hydrazine hydrate yielded the corresponding pyrazoline derivatives **4a** and **4b**. IR spectra of the prepared pyrazolines **4a** and **4b** showed υ (C=N) stretching at 1609–1604 cm^{-1} due to the ring closure. In addition, a sharp band at 3422–3393 cm^{-1} due to the υ (NH) stretching was also observed. ^{1}H-NMR spectra recorded for the prepared compounds in $CDCl_3$ clearly supported the proposed structures. Protons of pyrazoline ring in compounds **4a** and **4b** showed a prominent ABX system, with protons Ha, Hb, and Hx seen as doublets of doublets at δ 2.49–2.80, 3.39–3.81, and 5.00–5.10 ppm ($J_{Ha–Hb}$: 4.2–4.4, $J_{Ha–Hx}$: 7.4–8.2, $J_{Hb–Hx}$: 7.6 Hz), respectively. On the other hand, Hx of **4a** appeared as multiplet as a result of CH_3 substituent (Scheme 1).

Coupling of 3,4-disubstituted pyrazolines **4a** and **4b** with sulfonylated carbamic acid methyl esters **5a** and **5b** (Lange *et al.*, 2004) afforded **6a–d**. The structures of the prepared compounds were confirmed by the appearance of an additional strong absorption band of (C=O) stretching at υ 1683–1656 cm^{-1} in addition to absorption bands at υ 1380–1327 and 1163–1155 cm^{-1} that were attributed to (SO_2) stretching. Also, ^{1}H-NMR spectra showed an increase in the integration of the aromatic protons compared to the parent pyrazolines **4a** and **4b** and an additional singlet at δ 2.16–2.21 ppm corresponding to the three protons of the CH_3 in compounds **6a** and **6c** (Scheme 2).

Nucleophilic addition of pyrazoline derivatives **4a** and **4b** to 4-chlorobenzoyl isothiocyanate **7** (Cho and Shon, 1991) gave the adduct **8a** and **8b**. IR spectra of **8a** and **8b** revealed the presence of (C=O) at υ 1669 and 1656 cm^{-1}. ^{1}H-NMR of these compounds showed downfield shifting of the NH proton to δ 6.30 and 6.11 ppm (Scheme 3).

Reaction of propan-1-one derivative **2a** or phenyl ethanone derivative **2b** with diethyl oxalate in the presence of sodium hydride gave 4-aryl-3-substituted-2,4-dioxobutanoic acid ethyl esters **9a** and **9b**. IR spectra of these key intermediates **9a** and **9b** showed three strong absorption bands at υ 1665–1612 and 1730–1712 cm^{-1} (C=O of ketones and ester). ^{1}H-NMR of the 1,3-diketone structures **9a** and **9b** showed a signal for the CH proton that was observed as a quartet at nearly δ 6.00 ppm in **9a** or as a singlet at δ 6.40 ppm in **9b**, in addition to the appearance of ethyl protons at the expected chemical shifts (Scheme 4).

Condensation of **9a** and **9b** with hydrazine salts in ethanol and triethylamine afforded pyrazol-3-carboxylic acid ethyl esters **10a–d**. The structures of the prepared compounds **10a–d** were confirmed by the appearance of only one strong absorption band at υ 1738–1717 cm^{-1} corresponding to the carbonyl ester moiety. ^{1}H-NMR also confirmed the formation of the pyrazole ring from the disappearance of the characteristic signal for the CH proton that was observed in **9a** and **9b** (Scheme 4). Alkaline hydrolysis of the ester containing compounds **10b** and **10d** gave the corresponding free carboxylic acid derivatives **11a,b**. IR spectra showed a broad absorption band at υ 2950–2830 cm^{-1} for OH of the carboxylic group. ^{1}H-NMR confirmed the structures of the pyrazole derivatives **11a,b** from the appearance of an exchangeable proton at δ 11.00 ppm corresponding to COOH and disappearance of the characteristic pattern of the ethyl protons (Scheme 4). Mass spectra of the newly synthesized compounds were concomitant with their molecular weight.

Pharmacological evaluation

The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University.

LD_{50}

LD_{50} of each compound was determined using Finney's method (Finney, 1964). Intraperitoneal injection of the tested compounds in doses less than 75 mg/kg body weight failed to kill the mice within 24 h. LD_{50} celecoxib was 250 mg/kg body weight, and **2b**, **3a**, **4b**, **9a**, **9b**, **10a**, and **10d** were 277.5 mg/kg body weight. While of **4a**, **6c**, **8b**, and **10c** were 292.5 mg/kg body weight. LD_{50} of **6b**, **6d**, and **10b** was 247.5 mg/kg body weight, and **3b**, **6a**, **8a**, **11a**, and **11b** were 232.5 mg/kg body weight. As a result, the chosen dose was 25 mg/kg body weight which is similar to celecoxib and at the same time it is safe (Buck and Osweiter, 1976).

Anti-inflammatory activity (Table 1)

All the newly synthesized compounds **2–11** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema described by Winter *et al.* (Winter *et al.*, 1962). The tested compounds and reference drugs ibuprofen and celecoxib were intraperitoneal injected at a dose level of 25 mg/kg, 30 min before carrageenan injection at the right hind paw of albino male rats, the thickness of both paws was measured at different time intervals of 1, 2, 3, and 4 h after carrageenan injection. Anti-inflammatory activity of the tested compounds and reference drugs was calculated as the percentage decrease in edema thickness induced by carrageenan with the following formula (Alam *et al.*, 2009).

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

Table 1 Anti-inflammatory activity of ibuprofen, celecoxib, and the newly synthesized compounds (Carrageenan-induced edema in rats, $n = 5$)

Compound	Percent inhibition			
	1 h	2 h	3 h	4 h
Control	0	0	0	0
Ibuprofen	55.38 ± 1.97***	63.55 ± 2.41***	66.65 ± 1.85***	76.10 ± 2.12***
Celecoxib	54.21 ± 2.00***	61.07 ± 2.36***	63.97 ± 2.37***	62.75 ± 2.36***
2b	5.91 ± 2.15	11.23 ± 1.87	15.01 ± 1.74	15.92 ± 2.17
3a	23.66 ± 2.11*	29.78 ± 2.29*	31.50 ± 2.44**	32.34 ± 2.13**
3b	0	1.43 ± 2.24	1.92 ± 1.95	2.90 ± 1.67
4a	40.39 ± 2.39**	44.45 ± 2.72**	45.08 ± 2.54***	44.38 ± 2.53***
4b	0	0	1.80 ± 0.94	1.92 ± 0.94
6a	23.66 ± 1.96*	27.30 ± 1.82*	25.2 ± 2.23*	25.09 ± 1.93*
6b	12.25 ± 2.44	14.63 ± 2.33	16.45 ± 2.03	18.79 ± 2.33
6c	4.90 ± 2.28	6.33 ± 1.85	4.83 ± 1.97	7.21 ± 1.97
6d	24.15 ± 1.97*	27.30 ± 1.97*	25.69 ± 1.80*	23.62 ± 2.07*
8a	0	0	1.94 ± 0.94	1.43 ± 0.66
8b	0	0	1.43 ± 0.95	1.92 ± 0.92
9a	23.66 ± 1.96*	27.20 ± 2.12*	26.14 ± 2.24*	24.60 ± 2.08*
9b	15.05 ± 2.36	20.02 ± 2.22	20.82 ± 2.35	21.21 ± 2.35
10a	4.90 ± 2.43	4.86 ± 1.56	5.81 ± 1.97	5.77 ± 2.24
10b	0	1.43 ± 2.38	2.22 ± 1.43	2.38 ± 1.66
10c	17.74 ± 2.52	20.51 ± 2.08	20.82 ± 2.36	20.23 ± 2.05
10d	6.89 ± 2.29	12.70 ± 2.17	15.01 ± 2.31	15.43 ± 2.16
11a	24.69 ± 1.96*	28.35 ± 2.43*	22.75 ± 2.22*	22.68 ± 2.06*
11b	36.96 ± 2.20**	42.03 ± 2.86**	45.53 ± 2.71***	42.45 ± 2.35***

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

*** Significant at $P \leq 0.001$

where V_R represents the mean right paw thickness, V_L represents the mean left paw thickness, $(V_R - V_L)$ control represents the mean increase in paw thickness in the control group of rats, and $(V_R - V_L)$ treated represents the mean increase in paw thickness in rats treated with the tested compounds. The results listed in Table 1 revealed that some of the synthesized compounds showed moderate to weak anti-inflammatory activity. Compounds **4a** and **11b** induced considerable anti-inflammatory activity relative to ibuprofen, and celecoxib that reached to 67% after 3 h. Another important observation was the decline in activity of compounds **6d** and **8b** upon replacement of SO_2 by C=O. On the other hand, the triaryl carboxylic acid derivative **11b** was found to be twice as active as diaryl analog **11a**.

Analgesic activity (Table 2)

All the synthesized compounds as well as celecoxib were tested for their analgesic activity using the *p*-benzoquinone-induced writhing test in mice (Collier *et al.*, 1968). The results showed that compounds possessing good anti-inflammatory activity induced also good analgesic, except **10b** exhibited only analgesic activity (Table 2).

Table 2 Analgesic activity of celecoxib and the new synthesized compounds in mice (25 mg/kg body weight) using writhing method ($n = 5$)

Compound	Percent protection against writhing after (h)			
	1	2	3	4
Control	0	0	0	0
Celecoxib	100	100	80	80
3a	20	20	0	0
4a	80	60	40	20
6a	20	20	0	0
6d	40	40	20	0
10b	40	40	20	0
11a	40	20	20	0
11b	80	60	40	20

Ulcerogenic effect (Table 3)

The ulcerogenic effect of the most active compounds **3a**, **4a**, **11b**, and the reference drugs (ibuprofen, and celecoxib) was evaluated according to Meshali's method (Meshali *et al.*, 1983). The ulcer index was calculated according to Robert's method (Robert *et al.*, 1968). The results showed that compounds **3a**, **4a**, and **11b** exhibited little gastric ulceration; about 50–60% that of ibuprofen. On the other hand, these compounds showed higher gastric ulceration than celecoxib (Table 3).

Molecular modeling study

Molecular docking of celecoxib and the synthesized compounds were performed to rationalize the obtained biological results. Besides, molecular docking studies were helped in understanding the various interactions between the ligand and enzyme active site. Docking studies of the inhibitors were performed by MOE (Molecular Operating Environment, 2005) using murine COX-2 co-crystallized with celecoxib (PDB ID: 6COX) as a template. We performed 100 docking iterations for each ligand, and the top-scoring configuration of each of the ligand–enzyme complexes was selected on energetic grounds.

Table 3 Ulcerogenic effect of celecoxib, ibuprofen, **3a**, **4a**, and **11b** in rats (25 mg/kg body weight) ($n = 5$)

Compound	Percent incidence divided by ten	Average no. of ulcer	Average severity	Ulcer index
Control	0	0	0	0
Celecoxib	6	1.4	1.00	8.40
Ibuprofen	10	10.0	0.32	22.92
3a	10	4.2	1.04	15.24
4a	8	2.6	1.07	11.67
11b	8	2.4	1.16	11.56

Docking of celecoxib in the active site of murine COX-2 showed hydrogen bonds interactions between the SO_2 group and His90 (distances = 2.69 Å). Also, hydrogen bonds were observed between Gln192 and Leu352 with NH_2 group (distances = 3.01 and 3.43 Å, respectively). Ten hydrophobic bonds with Val116, Val349 (distance 3.70 Å), Leu352, Leu359, Phe381, Leu384, Try387, Phe518, and Val523 (distance 3.10 Å).

Docking of non pyrazole derivatives **2a**,**b**, **3a**,**b**, and **9a**,**b** revealed the appearance of hydrogen bonds between His90 and the 4-methoxy group of **2b**, **3b**, **9b**, or the carbonyl of the ester group of **9a** (distances = 3.48, 2.02, 1.73, and 3.39 Å). Also, hydrogen bonds were detected between Arg120 and 4-methoxy group of **2a** and **3a** (distances = 2.68 and 3.37 Å). In addition to, the presence of hydrophobic interactions especially with Val523, Leu352 (except **3a**), Val349 (except **2b**) and Phe518 for **2a**, **2b**, and **3a**.

Concerning 3-(2,4-dimethoxyphenyl)-4-methyl/phenyl-4,5-dihydro-1*H*-pyrazole **4a**,**b**, it was found that the hydrogen bonds were between Arg120, Leu352, and try355 with the 4-methoxy group, NH group, and 4-methoxy group for **4a** or 2-methoxy group for **4b**, respectively (distances = 2.64 and 2.68, 2.51 and 2.44, and 3.44 and 3.09 Å for **4a** and **4b**, respectively). The presence of the hydrophobic interactions with Val349, Val523 were observed, and an extra hydrophobic interaction with Leu352 was found in the case of **4b**.

For compounds **6a–d**, it was observed the presence of hydrogen bond between Arg120 and 4-methoxy group of compound **6d** (distances = 2.38 Å). On the other hand, hydrogen bonds were formed between His90 and SO_2 group of **6a**, **6b**, and **6c** or with the carbonyl group of **6d** (distances = 3.17, 2.60, 3.68, and 2.67 Å). Also, more hydrogen bonds were found between Arg513 and 4-methoxy group of them (distances = 2.51, 2.52, 3.07, and 3.07 Å for **6a–d**). Additional hydrogen bonds were observed between Leu352 and NH group of **6a**, **6b**, or between Ser530 and NH group of **6c**. Besides, the hydrophobic interactions with Val523, Val349, Phe518, Leu352 (except **6a**), Ile517 (for **6a**, **6b**) and Try387 (for **6c**).

Thiocarboxamide derivatives **8a**,**b** showed hydrogen bonds between Ser530 and NH group (distances = 2.98 and 2.63 Å). Another bond was detected between His90 and 4-methoxy group of **8b** (distance = 3.44 Å). In addition to, the hydrophobic interactions with Val523, Val349, Leu359, and Leu352.

Concerning compounds **10a–d** and **11a**,**b**, hydrogen bonds were appeared between Arg120 and 4-methoxy group of **10b**, **10d**, and **11a** (distances = 2.92, 2.86 and 2.75 Å) and between tyr355 and 4-methoxy group of **10a**,

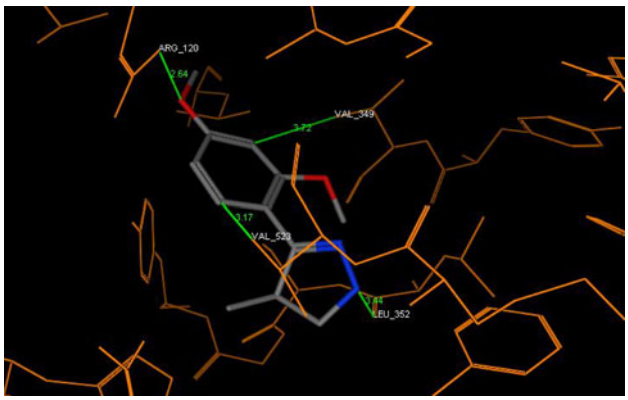


Fig. 1 3D Docked structure of compound **4a** in the active site of murine COX-2 showed two hydrogen bonds: 4-OCH_3 and Arg120 (2.64 Å) and pyrazole N and Leu352 (3.44 Å). Also, two hydrophobic bonds with Val349 and Val523 (3.72 and 3.17 Å, respectively)

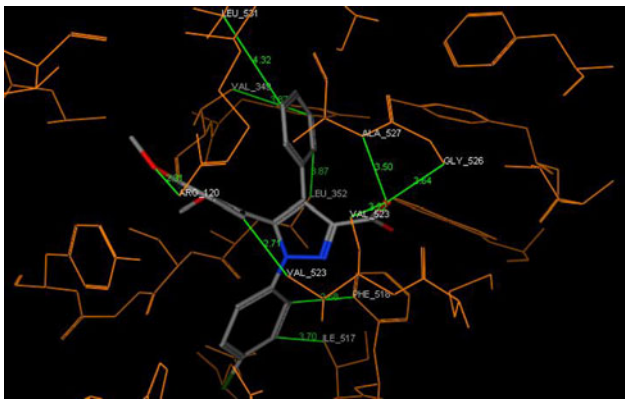


Fig. 2 3D Docked structure of compound **11b** in the active site of murine COX-2 showed four hydrogen bonds: Arg120, Val523, Gly526, and Ala527 (2.31, 3.22, 3.64, and 3.50 Å). Also, six hydrophobic bonds: Val349, Leu352, Ile517, Phe518, Val523, and Leu531 (3.87, 3.87, 3.70, 2.81, 2.71, and 4.32 Å, respectively)

10c or 2-methoxy group of **11b** (distances = 3.01, 2.78, and 2.68 Å). Also, the same pattern of the hydrophobic bonds with Val523 and Val349 (Figs. 1, 2).

Conformational alignment of celecoxib from the crystal structure of celecoxib-murine COX-2 complex and that of compound **4a** from the docking simulation is shown in Fig. 3. The superposition showed overlapping through heterocyclic and one aryl regions. While, that of compound **11b** is shown in Fig. 4 showed complete overlapping through heterocyclic and two aryl regions.

Conclusions

Various substituted pyrazole derivatives were synthesized and screened for anti-inflammatory, analgesic activities as well as for their ulcerogenic effect. Results showed that compounds **3a**, **4a**, **6a**, **6d**, **9a**, **11a**, and **11b** exhibited anti-inflammatory and analgesic activities with the exception of **10b** that established only analgesic activity.

SAR: Substitution of the pyrazole ring with at least one aryl moiety is essential for activity. Moreover, the presence of acidic center expressed by NH (of pyrazole ring) "compound **4a**" or (COOH) "compound **11b**" improves the anti-inflammatory activity. On the other hand, the ulcerogenic effect of **4a** and **11b** is slightly higher than that of celecoxib due to the primary insult effect.

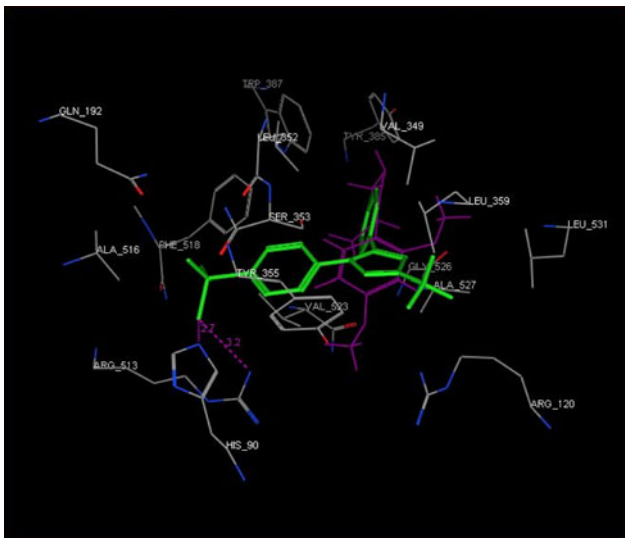


Fig. 3 Conformational alignment of celecoxib from the crystal structure of celecoxib-murine COX-2 complex and that of compound **4a** from the docking simulation

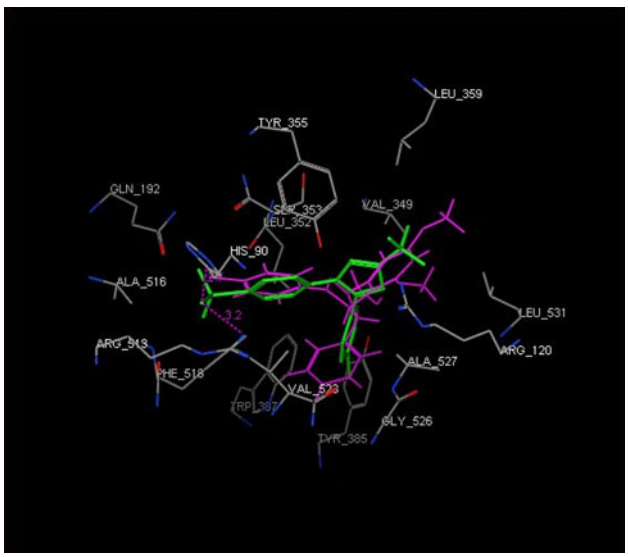


Fig. 4 Conformational alignment of celecoxib from the crystal structure of celecoxib-murine COX-2 complex and that of compound **11b** from the docking simulation

Experimental

Chemistry

All melting points were determined by the open capillary method using Gallen Kemp melting point apparatus (MFB-595-010M) and were uncorrected. Microanalyses were carried out at the Micro analytical Unit, Faculty of Science, and Cairo University. IR (KBr) was determined using Shimadzu Infrared Spectrometer (IR-435) and FT-IR 1650 (Perkin Elmer). ^{1}H-NMR Spectra were carried using Fourier transform EM-390, 200 MHz NMR Spectrometer and Varian Mercury VX-300 MHz NMR Spectrometer. Mass spectra were carried using Fining SSQ 7000 Gas Chromatograph Mass spectrometer and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX. Nomenclature of presented compounds was according to IUPAC system. Compounds **2a**, **5a**, and **5b** were prepared according to the literature procedures (Slotta and Heller, 1930; Lange *et al.*, 2004; Cho and Shon, 1991), respectively.

*1-(2,4-Dimethoxyphenyl)-2-phenyl ethanone **2b***

A solution of 1,3-dimethoxybenzene **1** (2.76 g, 20 mmol) and phenylacetic acid (2.72 g, 20 mmol) in polyphosphoric acid (50 ml) was heated in boiling water bath for 30 min. After cooling, the red residue was decomposed by pouring on ice-water and extracted with chloroform. The organic layer was washed twice with water, dried over Na_2SO_4, and concentrated. The residue was crystallized from MeOH to give **2b** (60% yield), mp 72–73°C. ^{1}H-NMR ($CDCl_3$) δ: 3.78 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2), 6.43 (s, 1H, ArH-3), 6.53 (m, 2H, Ar), 7.30 (m, 3H, Ar), 7.80 (d, 1H, ArH-5, *J*: 8.8 Hz), 8.12 (d, 1H, ArH-6, *J*: 8.8 Hz). IR (KBr) cm^{-1}: 3050 (CH Ar), 2964, 2939, 2834 (CH aliphatic), 1668 (C=O). MS *m/z*: 256 [M^+, 4%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{16}H_{16}O_3$ (256.20): C 74.98; H 6.29. Found: C 74.90; H 6.20.

*1-(2,4-Dimethoxyphenyl)-2-methyl prop-2-en-1-one **3a***

To a solution of 1-(2,4-dimethoxyphenyl) propan-1-one **2a** (1.95 g, 10 mmol) in MeOH (50 ml), piperdine (0.12 ml, 1.21 mmol), acetic acid (0.12 ml, 2.08 mmol), and formalin (4 ml: 37% aqueous solution, 5.32 mmol) were successively added, and the resulting mixture was refluxed for 4 h. The mixture was concentrated in vacuum. Water was added to the residue, and the mixture was extracted with chloroform. The organic layer was separated, washed with water (3×), dried over Na_2SO_4, filtered, and concentrated to give **3a** (70% yield) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.21(s, 3H, CH_3), 2.93 (d, 1H, CH, methylene, *J*: 7.4 Hz), 3.04 (d, 1H, CH, methylene, *J*: 7.4 Hz), 3.80 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.48 (s, 1H, ArH-3), 6.56 (d, 1H, ArH-5, *J*: 8.8 Hz), 7.86 (d, 1H, ArH-6, *J*: 8.4 Hz). IR (KBr) cm^{-1}: 3050 (CH Ar), 2934, 2840 (CH aliphatic), 1661 (C=O). MS *m/z*: 208 [$M^+ + 2$, 1%], 206 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{12}H_{14}O_3$ (206.24): C 69.89; H 6.84. Found: C 69.77; H 6.74.

*1-(2,4-Dimethoxyphenyl)-2-phenyl prop-2-en-1-one **3b***

Compound **3b** was prepared from **2b** as oil (60% yield) by the same procedure as described for **3a**. ^{1}H-NMR ($CDCl_3$) δ: 3.79 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.21 (d, 1H, CH, methylene, *J*: 8.2 Hz), 4.86 (d, 1H, CH, methylene, *J*: 8.2 Hz), 6.12 (s, 1H, ArH-3), 6.30–7.42 (m, 5H, Ar), 7.59 (d, 1H, ArH-5, *J*: 8.6 Hz), 8.31 (d, 1H, ArH-6, *J*: 8.6 Hz). IR (KBr) cm^{-1}: 3030 (CH Ar), 2930, 2840 (CH aliphatic), 1660 (C=O). MS *m/z*: 268 (M^+, 14%), 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{17}H_{16}O_3$ (268.31): C 76.10; H 6.01. Found: C 76.37; H 5.81.

*3-(2,4-Dimethoxypheny)-4-methyl-4,5-dihydro-1H-pyrazole **4a***

A solution of **3a** (2.06 g, 10 mmol), hydrazine hydrate (6.0 ml, 120 mmol) in absolute ethanol (50 ml) was refluxed for 3 h. The mixture was concentrated *in vacuum*; water was added to the residue and extracted with chloroform. The organic layer was twice washed with water, dried over Na_2SO_4, and concentrated. The residue was oil (70% yield). ^{1}H-NMR ($CDCl_3$-D_2O) δ: 1.20 (d, 3H, CH_3, *J*: 4.6 Hz), 2.49 (dd, 1H, CH, methylene, *J*: 4.2, 7.4 Hz), 3.81 (dd, 1H, CH, methylene, *J*: 42, 7.4 Hz), 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.00 (m, 1H, CH py), 5.29 (broad, 1H, NH exch.), 6.51 (s, 1H, ArH-3), 6.96 (d, 1H, ArH-5, *J*: 8.2 Hz), 7.45 (d, 1H, ArH-6, *J*: 8.2 Hz). IR (KBr) cm^{-1}: 3393 (NH), 3050 (CH Ar), 2936, 2836 (CH aliphatic), 1609 (C=N). MS *m/z*: 220 [M^+, 14%], 177 [C_6H_3 $(OCH_3)_2CH$=NNH, 100%]. Anal. calcd for $C_{12}H_{16}N_2O_2$ (220.27): C 65.43; H 7.32; N 12.72. Found: C 65.10; H 7.60; N 12.78.

*3-(2,4-Dimethoxypheny)-4-phenyl-4,5-dihydro-1H-pyrazole **4b***

Compound **4b** was prepared from **3b** as oil (75% yield) using the same procedure described for **4a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 2.80 (dd, 1H, CH, methylene, *J*: 4.4, 8.2 Hz), 3.39 (dd, 1H, CH, methylene, *J*: 4.4, 8.2 Hz), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.10 (d, 1H, CH *J*:7.6 Hz), 5.53 (s, 1H, NH exch.), 6.14 (s, 1H, ArH-3), 6.30–7.42 (m, 5H, Ar), 7.61 (d, 1H, ArH-5, *J*: 8.4 Hz), 8.31 (d, 1H, ArH-6, *J*: 8.4 Hz). IR (KBr) cm^{-1}: 3422 (NH), 3058 (CH Ar), 2934, 2837 (CH aliphatic), 1604 (C=N). MS *m/z*: 282 [M^+, 5%], 165 [$C_6H_3(OCH_3)_2CHNH$, 100%].

Anal. Calcd for $C_{17}H_{18}N_2O_2$ (282.34): C 72.32; H 6.43; N 9.92. Found: C 72.27; H 6.22; N 9.97.

3-(2,4-Dimethoxyphenyl)-N-[(4-methylphenyl)sulfonyl]-4-methyl-4,5-dihydro-1H-pyrazol-1-carboxamide ***6a***

To a solution of **4a** (2.20 g, 10 mmol) in toluene (50 ml), **5a** (2.75 g, 12 mmol) was added, and the resulting mixture was refluxed for 4 h. After cooling of the solution to room temperature, the mixture was concentrated *in vacuum*, water was added and extracted with chloroform. The organic layer was washed twice with water, dried over Na_2SO_4, and concentrated. The product was obtained as oil (80% yield). ^{1}H-NMR ($CDCl_3$–D_2O) δ: 1.21 (d, 3H, CH_3, *J*: 4.6 Hz), 2.21 (s, 3H, CH_3), 3.70 (d, 1H, CH, methylene, *J*: 4.4 Hz), 3.80 (d, 1H, CH, methylene, *J*: 4.4 Hz), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.11 (m, 1H, CH py), 5.27 (s, 1H, NH exch.), 6.51 (s, 1H, ArH-3), 7.20 (d, 1H, ArH-5, *J*: 8.4 Hz), 7.33 (d, 1H, ArH-6, *J*: 8.7 Hz), 7.80 (d, 2H, Ar, *J*: 8.4 Hz), 7.90 (d, 2H, Ar, *J*: 8.1 Hz). IR (KBr) cm^{-1}: 3328 (NH), 3030 (CH Ar), 2969, 2928 (CH aliphatic), 1683 (C=O), 1610 (C=N), 1327, 1155 (SO_2). MS *m/z*: 417 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CHNH$, 8%], 91 [C_7H_7, 100%]. Anal. calcd for $C_{20}H_{23}N_3O_5S$ (417.48): C 57.54; H 5.55; N 10.06. Found: C 57.40; H 5.77; N 9.96.

N-[(4-Chlorophenyl)sulfonyl]-3-(2,4-dimethoxyphenyl)-4-methyl-4,5-dihydro-1H-pyrazol-1-carboxamide ***6b***

Compound **6b** was prepared from **4a** and **5b** as white crystals from methanol, m.p. 225°C (78% yield) using the same procedure described for **6a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 1.45 (d, 3H, CH_3, *J*: 4.6 Hz), 3.07 (d, 1H, CH, methylene, *J*: 4.6 Hz), 3.16 (d, 1H, CH, methylene, *J*: 4.4 Hz), 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.60 (broad, 1H, CH), 4.78 (broad, 1H, NH exch.), 7.28 (s, 1H, ArH-3), 7.53 (d, 3H, Ar, *J*: 8.0 Hz), 7.91 (d, 3H, ArH, *J*: 8.2 Hz). IR (KBr) cm^{-1}: 3300 (NH), 3030 (CH Ar), 2900, 2800 (CH aliphatic), 1680 (C=O), 1600 (C=N), 1380, 1160 (SO_2). MS *m/z*: 423 [M^+-CH_3, 3%], 165 [C_6H_3 $(OCH_3)_2CHNH$, 48%], 57 [$C_2H_5N_2$, 100%]. Anal. calcd for $C_{19}H_{20}ClN_3O_5S$ (437.90): C 52.11; H 4.60; N 9.65. Found: C 52.18; H 5.20; N 9.57.

3-(2,4-Dimethoxyphenyl)-N-[(4-methylphenyl)sulfonyl]-4-phenyl-4,5-dihydro-1H-pyrazol-1-carboxamide ***6c***

Compound **6c** was prepared from **4b** and **5a** as oil (84% yield) using the same procedure described for **6a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 2.16 (s, 3H, CH_3), 3.60 (d, 1H, methylene, *J*: 4.0 Hz), 3.77 (d, 1H, methylene, *J*: 4.0 Hz), 3.82 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.20 (d, 1H, CH *J*:7.6 Hz), 5.30 (s, 1H, NH exch.), 6.20 (s, 1H, ArH-3), 6.60 (d, 1H, ArH-5, *J*: 8.4 Hz), 6.80 (d, 1H, ArH-6, *J*: 8.2 Hz), 7.00–7.65 (m, 5H, Ar), 7.77 (d, 2H, Ar, *J*: 8.0 Hz), 7.95 (d, 2H, Ar, *J*: 8.0 Hz). IR (KBr) cm^{-1}: 3261 (NH), 3063 (CH Ar), 2939, 2841 (CH aliphatic), 1660 (C=O), 1605 (C=N), 1343, 1162 (SO_2). MS *m/z*: 479 [M^+, 1%], 165 [C_6H_3 $(OCH_3)_2CHNH$, 100%]. Anal. calcd for $C_{25}H_{25}N_3O_5S$ (479.55): C 62.62; H 5.25; N 8.76. Found: C 62.62; H 5.25; N 8.83.

N-[(4-Chlorophenyl)sulfonyl]-3-(2,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1H-pyrazol-1-carboxamide ***6d***

Compound **6d** was prepared from **4b** and **5b** as oil (76% yield) adopting the same procedure described for **6a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 3.40 (d, 1H, methylene, *J*: 4.0 Hz), 3.60 (d, 1H, methylene, *J*: 4.0 Hz), 3.81 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 4.20 (d, 1H, CH *J*: 7.6 Hz), 5.20 (s, 1H, NH exch.), 6.15 (s, 1H, ArH-3), 6.605 (d, 1H, ArH-5, *J*: 8.2 Hz), 6.85 (d, 1H, ArH-6, *J*: 8.2 Hz), 7.00–7.70 (m, 5H, Ar), 7.88 (d, 2H, Ar, *J*: 8.4 Hz), 7.99 (d, 2H, Ar, *J*: 8.2 Hz). IR (KBr) cm^{-1}: 3363 (NH), 3090 (CH Ar), 2937, 2842 (CH aliphatic), 1656 (C=O), 1604 (C=N), 1346, 1163 (SO_2). MS *m/z*: 501 [M^++2, 1%], 499 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CHNH$, 100%]. Anal. calcd for $C_{24}H_{22}ClN_3O_5S$ (499.97): C 57.65; H 4.44; N 8.41. Found: C 57.91; H 4.62; N 8.48.

4-Chlorobenzoylisothiocynate ***7***

Compound **7** was prepared according to the reported procedure (Cho and Shon, 1991) from 4-chlorobenzoyl chloride and ammonium thiocyanate and immediately reacted with **4**.

N-(4-Chlorobenzoyl)-3-(2,4-dimethoxyphenyl)-4-methyl-4,5-dihydro-1H-pyrazol-1-thiocarboxamide ***8a***

Compound **4a** (2.20 g, 10 mmol) was added to a cold (0°C) solution of **7** (2.37 g, 12 mmol) in anhydrous acetonitrile (20 ml), and the resulting mixture was stirred at room temperature for 3 h. The precipitate was removed by filtration and thoroughly washed with acetonitrile. The filtrate was concentrated *in vacuum*, and the residue was collected and further purified by extraction with chloroform. The organic layer was twice washed with water, dried over Na_2SO_4, and concentrated. The product was obtained as oil (61% yield). ^{1}H-NMR ($CDCl_3$–D_2O) δ: 1.27 (d, 3H, CH_3), 3.20 (d, 1H, methylene, *J*: 4.2 Hz), 3.40 (d, 1H, methylene, *J*: 4.2 Hz), 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.49 (m, 1H, CH), 6.30 (s, 1H, NH exch.), 6.40(s, 1H, ArH-3), 7.26 (d, 1H, ArH-5, *J*: 8.6 Hz), 7.33 (d, 1H, ArH-6, *J*: 8.6 Hz), 7.76 (d, 2H, Ar, *J*: 8.8 Hz), 7.85 (d, 2H, Ar, *J*: 8.8 Hz). IR (KBr) cm^{-1}: 3360 (NH), 3050 (CH Ar), 2934, 2878 (CH aliphatic), 1669 (C=O), 1620 (C=N), 1098 (C=S). MS *m/z*:

417 [M^+, 2%], 385 [M^+-S, 3%], 177 [$C_6H_3(OCH_3)_2CNNH$, 100%]. Anal. calcd for $C_{20}H_{20}ClN_3O_3S$ (417.91): C 57.48; H 4.82; N 10.05. Found: C 57.68; H 4.84; N 10.43.

N-(4-Chlorobenzoyl)-3-(2,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1H-pyrazol-1-thiocarboxamide ***8b***

Compound **8b** was prepared from **4b** and **7** as oil (73% yield) by the same procedure as described for **8a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 3.20 (d, 1H, methylene, *J*: 4.2 Hz), 3.36 (d, 1H, methylene, *J*: 4.2 Hz), 3.89 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 4.59 (d, 1H, CH *J*: 7.5 Hz), 6.11 (s, 1H, NH exch.), 6.53 (s, 1H, ArH-3), 7.26–7.96 (m, 9H, Ar), 8.05 (d, 1H, Ar, *J*: 9.0 Hz), 8.35 (d, 1H, Ar, *J*: 8.6 Hz). IR (KBr) cm^{-1}: 3368 (NH), 3050 (CH Ar), 2853, 2772 (CH aliphatic), 1656 (C=O), 1619 (C=N), 1087 (C=S). MS *m/z*: 481 [M^++2, 35%], 479 [M^+, 1%], 281 [M^+–C_6H_4(*p*-Cl)CONHCS, 50%], 165 [$C_6H_3(OCH_3)_2C^+NH$, 100%]. Anal. calcd for $C_{25}H_{22}ClN_3O_3S$ (479.98): C 62.56; H 4.62; N 8.75. Found: C 62.50; H 4.60; N 8.76.

3-Methyl-2,4-dioxo-4-(2,4-dimethoxyphenyl) butanoic acid ethyl ester ***9a***

To compound **2a** (1.94 g, 10 mmol) in toluene (20 ml) was added sodium hydride (0.48 g, 20 mmol), and the mixture was stirred for 10 min. Then, diethyl oxalate (3.29 g, 3.0 ml, 15 mmol) was added dropwise, and the mixture was stirred at reflux (1 h). The mixture was cooled to room temperature, washed with ether, acidified with acetic acid, and extracted with chloroform. The organic layer was washed with water, and concentrated. The product was obtained as oil (60% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (t, 3H, $CH_2C\underline{H_3}$, *J*: 4.2 Hz), 2.35 (d, 3H, $COCHC\underline{H_3}$, *J*: 2.2 Hz), 3.79 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.30 (q, 2H, $C\underline{H_2}CH_3$), 6.00 (q, 1H, CH), 7.41 (s, 1H, ArH-3), 7.66 (d, 1H, ArH-5, *J*: 8.0 Hz), 8.08 (d, 1H, ArH-6, *J*: 8.0 Hz). IR (KBr) cm^{-1}: 3030 (CH Ar), 2969, 2843 (CH aliphatic), 1712, 1612 (3 C=O). MS *m/z*: 417 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CHNH$, 8%], 91 [C_7H_7, 100%]. Anal. calcd. for $C_{15}H_{18}O_6$ (294.31): C 61.22; H 6.12. Found: C 61.49; H 5.88.

2,4-Dioxo-3-phenyl-4-(2,4-dimethoxyphenyl) butanoic acid ethyl ester ***9b***

Compound **9b** was prepared from **2b** as oil (72% yield) following the same procedure described for **9a**. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (t, 3H, $CH_2C\underline{H_3}$, *J*: 7.2 Hz), 3.77 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.28 (q, 2H, $C\underline{H_2}CH_3$), 6.44 (s, 2H, COCH & ArH-3), 6.54 (d, 1H, ArH-5, *J*: 8.6 Hz), 7.15–7.60 (m, 5H, Ar), 7.83 (d, 1H, ArH-6, *J*: 8.4 Hz). IR (KBr) cm^{-1}: 3060 (CH Ar), 2940, 2838 (CH aliphatic), 1730, 1665 (3 C=O). MS *m/z*: 417 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CHNH$, 8%], 91 [C_7H_7, 100%]. Anal. calcd for $C_{20}H_{20}O_6$ (356.38): C 67.41; H 5.66. Found: C 67.69; H 5.40.

5-(2,4-Dimethoxyphenyl)-4-methyl-1-phenyl-1H-pyrazole-3-carboxylic acid ethyl ester ***10a***

To a solution of **9a** (2.94 g, 10 mmol) in absolute ethanol (50 ml), phenyl hydrazine hydrochloride (1.45 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) were added. The reaction mixture was then heated under reflux for 9 h. It was then cooled, washed with water, and extracted with chloroform (3× 20 ml). The organic layer was then separated, dried over sodium sulfate, and evaporated to give **10a**. The product was oil (84% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (t, 3H, $CH_2C\underline{H_3}$, *J*: 4.0 Hz), 2.96 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.20 (q, 2H, $C\underline{H_2}CH_3$), 6.42 (s, 1H, ArH-3), 6.60 (d, 1H, ArH-5, *J*: 8.4 Hz), 7.10–7.80 (m, 5H, Ar), 8.11 (d, 1H, ArH-6, *J*: 8.4 Hz). IR (KBr) cm^{-1}: 3061 (CH Ar), 2935, 2843 (CH aliphatic), 1717 (C=O). MS *m/z*: 366 [M^+, 2%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{21}H_{22}N_2O_4$ (366.42): C 68.84; H 6.05; N 7.68. Found: C 68.65; H 6.24; N 7.59.

1-(4-Chlorophenyl)-5-(2,4-dimethoxyphenyl)-4-methyl-1H-pyrazole-3-carboxylic acid ethyl ester ***10b***

Compound **10b** was prepared from **9a** (2.94 g, 10 mmol), 4-chlorophenyl hydrazine hydrochloride (1.79 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) following the same procedure described for **10a**. The product was obtained as oil (80% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, 3H, $CH_2C\underline{H_3}$, *J*: 4.0 Hz), 2.92 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.34 (q, 2H, $C\underline{H_2}CH_3$), 6.38 (s, 1H, ArH-3), 6.43 (d, 1H, ArH-5, *J*: 8.2 Hz), 7.40 (d, 1H, ArH-6, *J*: 8.2 Hz), 7.80 (d, 2H, Ar, *J*: 8.4 Hz), 8.06 (d, 2H, Ar, *J*: 8.4 Hz). IR (KBr) cm^{-1}: 3050 (CH Ar), 2933, 2843 (CH aliphatic), 1720 (C=O). MS *m/z*: 402 [M^++2, 1%], 400 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{21}H_{21}ClN_2O_4$ (400.98): C 62.90; H 5.28; N 7.02. Found: C 63.14; H 5.46; N 7.32.

5-(2,4-Dimethoxyphenyl)-1,4-diphenyl-1H-pyrazole-3-carboxylic acid ethyl ester ***10c***

It was prepared from **9b** (3.56 g, 10 mmol), phenyl hydrazine hydrochloride (1.45 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) using the same procedure described for **10a**. The product was obtained as oil (74% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (t, 3H, $CH_2C\underline{H_3}$, *J*: 4.2 Hz), 3.78 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.30 (q, 2H, $C\underline{H_2}CH_3$), 6.23 (s, 1H, ArH-3), 6.40 (d, 1H, ArH-5, *J*:

8.2 Hz), 6.80–8.00 (m, 11H, Ar). IR (KBr) cm^{-1}: 3057 (CH Ar), 2927, 2843 (CH aliphatic), 1738 (C=O). MS *m/z*: 428 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{26}H_{24}N_2O_4$ (428.49): C 72.88; H 5.65; N 6.54. Found: C 72.87; H 5.30; N 6.25.

1-(4-Chlorophenyl)-5-(2,4-dimethoxyphenyl)-4-phenyl-1H-pyrazole-3-carboxylic acid ethyl ester ***10d***

It was prepared from **9b** (3.56 g, 10 mmol), 4-chlorophenyl hydrazine hydrochloride (1.79 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) following the same procedure described for **10a**. The product was obtained as oil (88% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (t, 3H, $CH_2\underline{CH_3}$, *J*: 4.0 Hz), 3.79 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.28 (q, 2H, $C\underline{H_2}CH_3$), 6.30 (s, 1H, ArH-3), 6.45 (d, 1H, ArH-5, *J*: 8.2 Hz), 6.65 (d, 1H, ArH-6, *J*: 8.2 Hz), 7.00–7.60 (m, 5H, Ar), 7.80 (d, 2H, Ar, *J*: 8.6 Hz), 8.10 (d, 2H, Ar, 8.6 Hz). IR (KBr) cm^{-1}: 3059 (CH Ar), 2937, 2839 (CH aliphatic), 1728 (C=O). MS *m/z*: 464 [M^++2, 1%], 462 [M^+, 1%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{26}H_{23}ClN_2O_4$ (462.93): C 67.46; H 5.01; N 6.05. Found: C 67.44; H 5.21; N 5.83.

1-(4-Chlorophenyl)-5-(2,4-dimethoxyphenyl)-4-methyl-1H-pyrazole-3-carboxylic acid ***11a***

Compound **10b** (2.00 g, 5 mmol) was dissolved in methanol (20 ml) and to this a solution of KOH (0.56 g, 10 mmol) in methanol (5 ml) was added. The reaction mixture was heated to reflux for 3 h, cooled, and poured into ice-water. It was acidified with acetic acid and extracted with chloroform. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was obtained as oil (51% yield). ^{1}H-NMR ($CDCl_3$–D_2O) δ: 3.08 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.23 (s, 1H, ArH-3), 6.50 (d, 1H, ArH-5, *J*: 8.0 Hz), 7.10 (d, 1H, ArH-6, *J*: 8.0 Hz), 7.35 (d, 2H, Ar, *J*: 8.6 Hz), 7.78 (d, 2H, Ar, *J*: 8.6 Hz), 11.00 (s, 1H, COOH, exch.). IR (KBr) cm^{-1}: 3030 (CH Ar), 2950–2850 (CH aliphatic and OH), 1720 (C=O). MS *m/z*: 372 [M^+, 8%], 165 [$C_6H_3(OCH_3)_2CO$, 100%]. Anal. calcd for $C_{19}H_{17}ClN_2O_4$ (372.81): C 61.21; H 4.60; N 7.51. Found: C 61.35; H 4.80; N 7.57.

1-(4-Chlorophenyl)-5-(2,4-dimethoxyphenyl)-4-phenyl-1H-pyrazole-3-carboxylic acid ***11b***

It was prepared from **10d** as oil (57% yield) by using the same procedure described for **11a**. ^{1}H-NMR ($CDCl_3$–D_2O) δ: 3.83 (s, 3H, OCH_3), 4.27 (s, 3H, OCH_3), 6.35 (s, 1H, ArH-3), 6.50 (d, 1H, ArH-5, *J*: 8 Hz), 6.62 (d, 1H, ArH-6, *J*: 8.2 Hz), 7.60–7.78 (m, 5H, Ar), 7.88 (d, 2H, Ar, *J*: 8.4 Hz), 8.20 (d, 2H, ArH, *J*: 8.7 Hz), 11.00 (s, 1H, COOH, exch.). IR (KBr) cm^{-1}: 3060 (CH Ar), 2940–2830 (CH aliphatic and OH), 1730 (C=O). MS *m/z*: 436 [M^++2, 56%], 434 [M^+, 20%], 151 [$C_6H_3(OCH_3)_2CH_2$, 100%]. Anal. calcd for $C_{24}H_{19}ClN_2O_4$ (434.88): C 66.29; H 4.40; N 6.44. Found: C 66.41; H 4.79; N 6.07.

Pharmacological evaluation

The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University.

LD_{50}, anti-inflammatory, and analgesic activities of all the newly synthesized compounds were performed. The tested compounds were dissolved in dimethyl sulfoxide and water.

Determination of LD_{50}

LD_{50} determined using Finney's method (Finney, 1964). For this purpose, albino mice (25–30 g) were divided into groups each of five animals. Preliminary experiments were done for each compound to determine the minimal dose that kills all mice and the maximal dose that fails to kill any animal. Several increasing intraperitoneal doses were given in between these doses. Animals were kept under observation for 24 h during which symptoms of toxicity and rate of mortality in each group were recorded.

Anti-inflammatory activity (in vivo screening, carrageenan-induced edema in rats)

The anti-inflammatory activity of the newly synthesized compounds was evaluated according to the method described by Winter *et al*. (Winter *et al*., 1962). One-hundred and five rats of both sexes weighing 150–180 g were divided into 21 groups. The thickness of the left hind paw of each rat was measured in millimeter using a vernier caliber. The first group was kept as a control, while the second was intraperitoneal injected with celecoxib as a reference in a dose of 25 mg/kg body weight. Other groups were intraperitoneal injected with the tested compounds in a dose 25 mg/kg body weight which nearly equal to 1/10 their LD_{50}. After 30 min, inflammation was induced by subcutaneous injection of 50 µl of 1% carrageenan in a normal saline into the left hind paw. Degree of inflammation (mm) is measured as the difference between thickness after carrageenan treatment and thickness before carrageenan treatment. The paw thickness was measured hourly for a period of 4 h. The anti-inflammatory efficacy of the tested compounds was assessed by comparing the magnitude of the paw swelling in the treated animals with that of the

control then calculates percent inhibition with the following formula (Alam *et al.*, 2009).

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

Analgesic activity

The analgesic activity of the tested compounds was evaluated using the writhing method (Collier *et al.*, 1968). One-hundred and five mice of both sexes weighing 25–30 g were divided into 21 groups. The first group was kept as a control, while the second was intraperitoneal injected with celecoxib as standard drug in a dose of 25 mg/kg body weight. Other groups were intraperitoneal injected with the tested compounds in a dose 25 mg/kg body weight. After 30 min, each mouse was intraperitoneal injected with 0.25 ml of *p*-benzoquinone aqueous solution (0.1 mg/ml). Thereafter, mice in all groups were observed for writhing hourly for 4 h. Animals devoid of writhing in each group were counted, and the analgesic potency of the tested compounds was determined as percent protection against writhing.

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

Ulcerogenic effect

The ulcerogenic effect of the most active compounds **3a**, **4a**, and **11b** as well as both celecoxib and ibuprofen was evaluated according to Meshali's method (Meshali *et al.*, 1983). Thirty adult male albino rats weighing 120–150 g were used in this study. Animals were divided into six groups and received the drug orally. The first group received 2% tween 80 and kept as control, while the second and third groups received celecoxib and ibuprofen in a dose of 25 mg/kg body weight. The other groups were received **3a**, **4a**, and **11b** in the same dose. Animals were fed 2 h after administration of the drug. Rats received the given dose orally for three successive days. Two hours following the last dose, rats were sacrificed; the stomach of each rat was removed, opened along the greater curvature, and rinsed with 0.9% sodium chloride (isotonic solution). The stomach was stretched, by pins, on a corkboard. Examination with a magnifying lens (10×) was done for the presence of ulcers and erosions. The ulcer index was calculated according to Robert's method (Robert *et al.*, 1968). The degree of ulcerogenic effect was expressed in term of the percentage incidence of ulcers in each group of animals divided by ten, the average number of ulcers per stomach, and the average severity of ulcers by visual observation. The ulcer index is the value that result from the sum of the above three values.

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