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RESEARCH ARTICLE

Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of new 1,5-diarylpyrazole derivatives

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Abstract

A new series of 1,5-diarylpyrazoles **10a–l** was designed and synthesized for evaluation as COX inhibitors and as anti-inflammatory agents. All compounds were more selective for COX-2 isozyme and showed good *in vivo* anti-inflammatory activity. Compound **10e** was the most COX-2 selective compound (S.I. = 10.67) and the most potent anti-inflammatory derivative (ED_{50} = 46 μmol/kg) which is approximately 11-folds more potent than ibuprofen (ED_{50} = 499 μmol/kg) and had 2/3 potency of celecoxib (ED_{50} = 31 μmol/kg). All compounds were less ulcerogenic (ulcer indexes = 1.20–4.61) than ibuprofen (ulcer index = 20.25) and comparable to celecoxib (ulcer index = 2.90).

Keywords

Anti-inflammatory, cyclooxygenase-2 inhibitors, 1,5-diarylpyrazole

History

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Introduction

Inflammation is a multi-staged process whose critical phase is thought to be driven by acutely released arachidonic acid and its metabolites including prostaglandins[1]. Two cyclooxygenase (COX) isozymes, COX-1 and COX-2 are known to catalyze the rate limiting step of prostaglandin synthesis[2]. Non-steroidal anti-inflammatory drugs (NSAIDs) have become the most widely used pharmaceuticals for treatment of inflammation and pain by blocking the action of both COX isoforms[3]. COX-2 is upregulated by inflammatory mediators and forms prostaglandins which intensify the inflammatory response while COX-1 is the house keeping isozyme making prostaglandins which are important for maintaining physiological functions in the body[4]. Traditional NSAIDs as aspirin, ibuprofen and indomethacin exert their anti-inflammatory (AI) effect through inhibition of both COX-1 and COX-2 and in turn their use was accompanied with adverse effects including gastric bleeding and ulceration[5]. Thus, it was thought that selective COX-2 inhibitors would have reduced side effects with improved gastric safety profile. Most of the selective COX-2 inhibitors (Figure 1) (coxibs) are diarylheterocycles in which, two vicinal (adjacent) aryl moieties are attached to a five-membered ring as pyrazole in celecoxib (**1**)[6], furanone in rofecoxib (**2**)[7] or isoxazole in valdecoxib (**3**)[8]. Also, one of the two aryl rings is substituted at para position with one COX-2 pharmacophoric moiety either sulfamoyl (SO_2NH_2) or methanesulfonyl (SO_2CH_3) moiety[9,10]. Realization about the importance of COX-2 selective inhibitors for decreasing side effects associated with nonselective COX inhibitors has stimulated our group for designing and synthesis of new series of diarylpyrazolines **4**, **5** and diarylpyrazoles **6**, **7** of moderate COX-2 selectivity and good safety profile[11–13].

Based on the aforementioned information, we now describe the synthesis, *in vitro* evaluation as COX-1/COX-2 inhibitors, *in vivo* AI activity and ulcerogenic liability for a new series of diarylpyrazoles **10a–l** which maintains the vicinal diarylheterocycle scaffold and is closely related to the structure of celecoxib (**1**) but with three modifications: (i) the tolyl moiety of celecoxib was replaced with electron withdrawing moieties as (4-bromophenyl in **10a–c**, 4-nitrophenyl in **10d–f** or 4-chlorophenyl in **10g–i**) or replaced with another electron donating moiety (2-thienyl in **10j–l**) to check the effect of these substituents on the biological activity, (ii) trifluoromethyl moiety at C-3 of the central five-membered pyrazole ring was removed since it was reported that the substituent at C-3 of the central ring has very few steric restrictions with respect to COX-2 binding[14] and (iii) The COX-2 pharmacophore SO_2NH_2 was maintained (**10a**, **10d**, **10g** and **10j**), replaced with another COX-2 pharmacophore SO_2CH_3 (**10b**, **10e**, **10h** and **10k**) or replaced with COOH (**10c**, **10f**, **10i** and **10l**) (Figure 2).

Results and discussion

Chemistry

The enaminone derivatives (**8a–d**) were prepared *via* condensation of the appropriate aryl or heteroaryl methyl ketone with dimethylformamide dimethylacetal (DMFDMA) in xylene following procedures applied in literatures[15–21]. Cyclocondensation of the appropriate enaminone (**8a–d**) with 4-hydrazinylbenzenesulfonamide hydrochloride (**9a**)[22], methanesulfonylphenylhydrazine hydrochloride (**9b**)[23,24] or 4-hydrazinobenzoic acid (**9c**)[25] in aqueous ethanol afforded the respective 1,5-diarylpyrazoles (**10a–l**) in good yields (56–88%) (Scheme 1).

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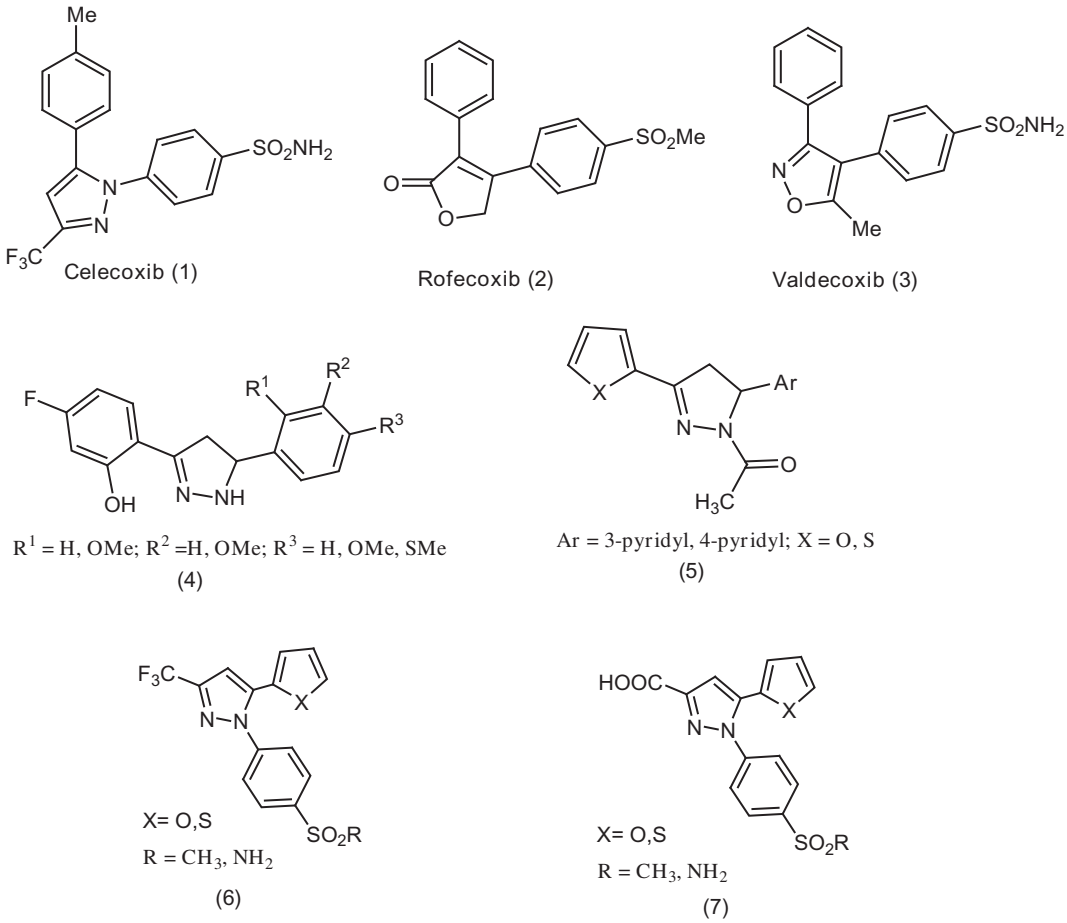
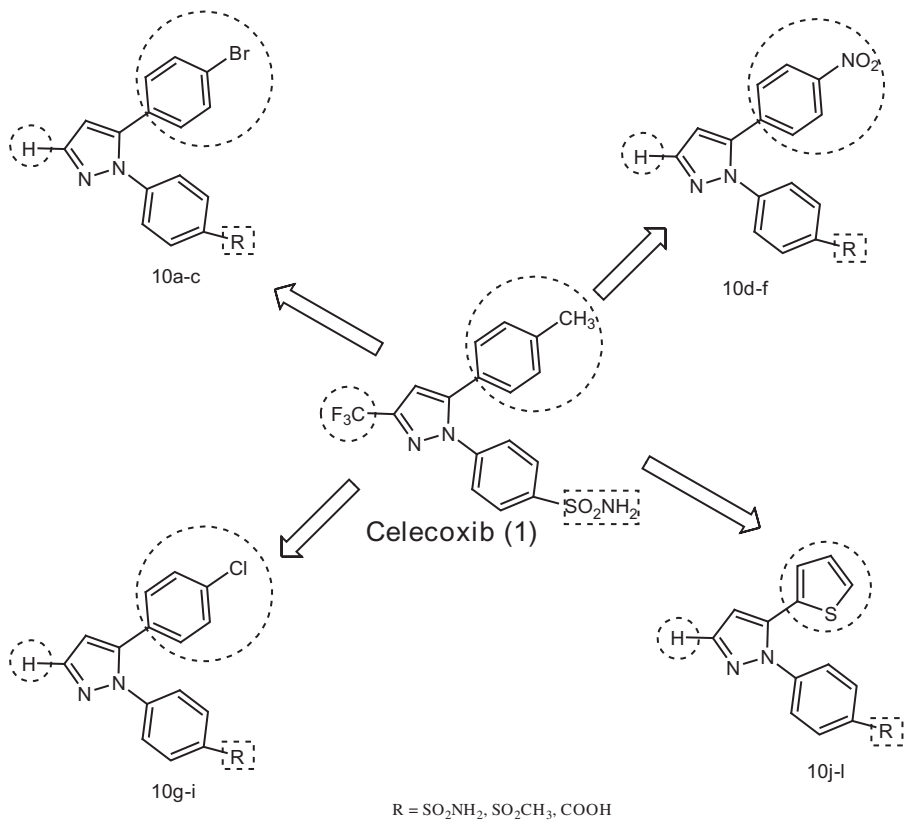
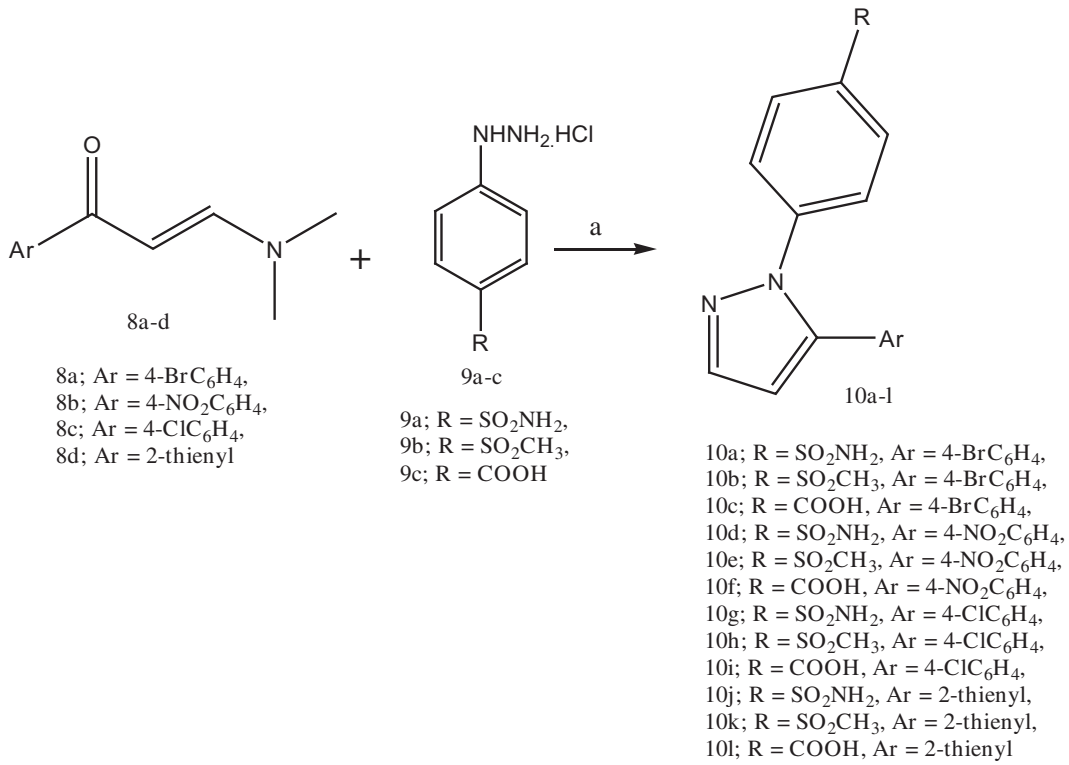


Figure 1. Chemical structures of the selective cyclooxygenase-2 inhibitors celecoxib (**1**), rofecoxib (**2**), valdecoxib (**3**), diarylpyrazoline derivatives (**4**, **5**) and the diarylpyrazoles (**6**, **7**).

Figure 2. Chemical structures of the selective cyclooxygenase-2 inhibitor celecoxib (**1**) and the designed dihydropyrazoles **10a**–**l**.





Scheme 1. Reagents and conditions: (a) EtOH (95%), reflux, 36 h.

Biological evaluation

In vitro *cyclooxygenase inhibition assay*

The *in vitro* COX-1/COX-2 isozyme inhibition studies measure the ability of tested compounds to inhibit ovine COX-1 and human recombinant COX-2 using an enzyme immunoassay (EIA)[26]. The results (Table 1) showed that all the diarylpyrazoles (**10a–l**) are week inhibitors for COX-1 isozyme ($IC_{50} = 2.91–10.21\ \mu M$ range) and exhibited moderate COX-2 isozyme inhibitory activities ($IC_{50} = 0.33–3.41\ \mu M$ range) with COX-2 selectivity indexes in the range of 2.79–10.67. While the 4-nitrophenyl analogs (**10d–f**) had the highest COX-2 potency ($IC_{50} = 0.33–3.41\ \mu M$ range) and the highest COX-2 selectivity indexes (S.I. = 8.56–10.67), the other analogs (4-bromophenyl analogs **10a–c**, 4-chlorophenyl analogs **10g–i** and 2-thienyl analogs **10j–l**) had lower COX-2 potency ($IC_{50} = 1.43–2.89$, 2.24–2.98 and 1.52–3.41 μM ranges respectively) and lower COX-2 selectivity indexes (S.I. = 2.91–4.80, 2.79–3.42 and 2.84–3.93 ranges, respectively). Within the all derivatives (**10a–l**), the 4-nitrophenylmethanesulphonylphenyl derivative (**10e**) had the highest COX-2 selectivity index (S.I. = 10.67) which is also higher than that of the COX-2 selective reference drug celecoxib (S.I. = 9.29). Also, for each group of compounds; while the COOH derivative (**10c**) was the most COX-2 selective (S.I. = 4.80) for the 4-bromophenyl analogs (**10a–c**) and the SO_2CH_3 derivative (**10e**) was the most COX-2 selective (S.I. = 10.67) for the 4-nitrophenyl analogs (**10d–f**), the SO_2NH_2 derivatives (**10g**, S.I. = 3.42, **10j**, S.I. = 3.93) were the most COX-2 selective derivatives for 4-chlorophenyl analogs **10g–i** and 2-thienyl analogs **10j–l**, respectively.

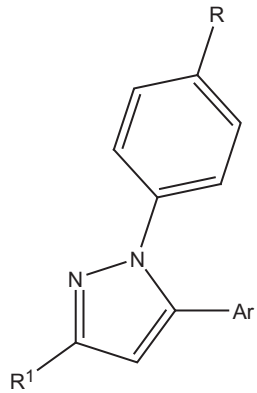
In vivo *anti-inflammatory activity*

The AI activities exhibited by the synthesized compounds were determined using a carrageenan-induced rat paw edema model and the dose causing 50% edema inhibition (ED_{50}) was determined in comparison to the reference drugs celecoxib and ibuprofen[28]. The tested diarylpyrazoles (**10a–l**) exhibited a broad AI activity range ($ED_{50} = 46–878\ \mu mol/kg$) in comparison with reference drugs celecoxib ($ED_{50} = 31\ \mu mol/kg$) and ibuprofen ($ED_{50} = 499\ \mu mol/kg$). Similar to the *in vitro* results, while the 4-nitrophenyl analogs (**10d–f**) had the highest AI activities ($ED_{50} = 46–148\ \mu mol/kg$ range), the other analogs (4-bromophenyl analogs **10a–c**, 4-chlorophenyl analogs **10g–i** and 2-thienyl analogs **10j–l**) had lower AI activities ($ED_{50} = 465–758$, 503–737 and 305–878 μmol/kg ranges respectively). Also, within the all derivatives (**10a–l**), the 4-nitrophenyl-methanesulphonylphenyl derivative (**10e**), the most COX-2 selective derivative, was the most potent derivative ($ED_{50} = 46\ \mu mol/kg$) which is approximately 11-folds more potent than ibuprofen ($ED_{50} = 499\ \mu mol/kg$) and 2/3 potency of celecoxib ($ED_{50} = 31\ \mu mol/kg$).

Ulcerogenic liability

The target compounds **10a–l** were subjected to further study to determine their ulcerogenic effect (ulcer index) using ED_{50} dose in comparison with celecoxib (ED_{50} dose) and small dose of Ibuprofen ($120\ \mu mol/kg$)[29]. The results revealed that all compounds **10a–l** were less ulcerogenic (ulcer indexes = 1.20–4.61) than ibuprofen (ulcer index = 20.25) and comparable to celecoxib (ulcer index = 2.90) (Table 2). Also, it was clear that, within each group of analogs, the carboxylic derivative was more ulcerogenic than the other derivatives which could be correlated to local acidity effect of their carboxylic group. For 4-bromophenyl analogs **10a–c**, the carboxylic derivative **10c** (ulcer index = 4.61) while the sulphamoyl derivative **10a** and the methanesulphonyl derivative **10b** had ulcer indexes = 2.92 and 2.68, respectively. Similarly, within 4-nitrophenyl analogs **10d–f**, 4-chlorophenyl analogs **10g–i** and 2-thienyl analogs **10j–l** the carboxylic derivatives **10f**, **10i** and **10l** (ulcer indexes = 4.32, 4.36 and 3.89 respectively) while the sulphamoyl derivatives **10d**, **10g** and **10j** (ulcer indexes = 1.20, 3.03 and 2.63, respectively) and the

Table 1. *In vitro* COX-1, COX-2 inhibition, anti-inflammatory activity of diarylpyrazoles (**10a–l**), and reference drugs celecoxib and ibuprofen.



10a-l

Compound	R	R^1	Ar	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	COX-2 S.I.[b]	ED_{50} (μmol/kg)[c]
10a	SO_2NH_2	H	4-BrC_6H_4	7.52	2.33	3.22	465
10b	SO_2CH_3	H	4-BrC_6H_4	8.42	2.89	2.91	604
10c	COOH	H	4-BrC_6H_4	6.87	1.43	4.80	758
10d	SO_2NH_2	H	4-$NO_2C_6H_4$	3.51	0.41	8.56	145
10e	SO_2CH_3	H	4-$NO_2C_6H_4$	5.87	0.55	10.67	46
10f	COOH	H	4-$NO_2C_6H_4$	2.91	0.33	8.81	148
10g	SO_2NH_2	H	4-ClC_6H_4	7.67	2.24	3.42	525
10h	SO_2CH_3	H	4-ClC_6H_4	8.11	2.63	3.08	737
10i	COOH	H	4-ClC_6H_4	8.34	2.98	2.79	503
10j	SO_2NH_2	H	2-thienyl	5.98	1.52	3.93	878
10k	SO_2CH_3	H	2-thienyl	9.23	3.25	2.84	305
10l	COOH	H	2-thienyl	10.21	3.41	2.99	537
Celecoxib	SO_2NH_2	CF_3	4-$CH_3C_6H_4$	7.34	0.79	9.29	31
Ibuprofen[d]	–	–	–	2.9	1.1	2.64	499

[a]The concentration of test compound produce 50% inhibition of COX-1, COX-2 enzyme, the result is the mean of two value obtained by assay of enzyme kits obtained from (Cayman Chemicals Inc., Ann Arbor, MI).
[b]The *in vitro* COX-2 selectivity index (COX-1/COX-2).
[c]Inhibitory activity in a carrageenan induced rat paw edema assay. The results are expressed as the ED_{50} value (μmol/kg) at 3 h after oral administration of the test compound.
[d]Data quoted from literature[28].

Table 2. Ulcerogenic liability for diarylpyrazoles (**10a**–l) and reference drugs celecoxib and ibuprofen.

Compound	Average severity	Average no. of ulcer[a]	% incidence/10	Ulcer index
10a	$0.73 \pm 0.017^{***,b}$	$0.2 \pm 0.004^{***,b}$	2	2.92
10b	$0.38 \pm 0.016^{***,b}$	$0.3 \pm 0.001^{***,b}$	2	2.68
10c	$1.2 \pm 0.037^{***,b}$	$0.4 \pm 0.016^{***,b}$	3	4.61
10d	$0.1 \pm 0.002^{***,b}$	$0.1 \pm 0.003^{***,b}$	1	1.2
10e	$0.28 \pm 0.015^{***,b}$	0.2 ± 0.007^{b}	2	2.47
10f	1.00 ± 0.040^{b}	$0.3 \pm 0.008^{***,b}$	3	4.32
10g	$0.83 \pm 0.030^{***}$	$0.2 \pm 0.007^{***,b}$	2	3.03
10h	$0.62 \pm 0.026^{***,b}$	$0.4 \pm 0.015^{***,b}$	2	3.02
10i	$0.85 \pm 0.035^{***,b}$	0.5 ± 0.012,b	3	4.36
10j	$0.25 \pm 0.009^{***,b}$	0.4 ± 0.006,b	2	2.63
10k	$0.17 \pm 0.004^{***,b}$	$0.5 \pm 0.012^{***,b}$	2	2.66
10l	$0.57 \pm 0.013^{***,b}$	$0.3 \pm 0.004^{***,b}$	3	3.89
Celecoxib	0.5 ± 0.013^{b}	0.4 ± 0.006^{b}	2	2.9
Ibuprofen	2.25 ± 0.13	8	10	20.25

***Significant difference with celecoxib at $p < 0.001$.
[a]Values represent means ± SEM of 10 animals for each group.
[b]Significant difference with ibuprofen at $p < 0.001$.

methanesulphonyl derivatives **10e**, **10h** and **10k** (ulcer indexes = 2.47, 3.02 and 2.66 respectively). The sulphamoyl derivative **10d** was the most safe derivative (ulcer index = 1.20) with relative ulcerogenicities to the reference drugs celecoxib and ibuprofen 0.41 and 0.06, respectively.

Conclusion

A new series of 1,5-diarylpyrazoles **10a–l** was synthesized for evaluation as selective COX-2 inhibitors, AI agents and ulcerogenic liability. Structure-activity data acquired and biological studies showed that (i) all compounds were more COX-2

inhibitors than COX-1, (ii) the 4-nitrophenyl analogs (**10d–f**) had higher AI activities than the other analogs (4-bromophenyl analogs **10a–c**, 4-chlorophenyl analogs **10g–i** and 2-thienyl analogs **10j–l**), (iii) the 4-nitrophenyl-methanesulphonylphenyl derivative (**10e**), the most COX-2 selective derivative, was the most potent derivative which is approximately 11-folds more potent than ibuprofen ($ED_{50} = 499$ μmol/kg) and 2/3 potency of celecoxib ($ED_{50} = 31$ μmol/kg), and (iv) all compounds were less ulcerogenic than ibuprofen and showed ulceration effect comparable to that of celecoxib.

Experimental

Chemistry

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on KBr plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz NMR Spectrophotometer, Faculty of Pharmacy, Beni-Suef University, Egypt in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz). Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the electro-spray (ES) ionization mode. Microanalyses were performed for C, H and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT) at the micro analytical unit of Cairo University, Egypt. All compounds were within ±0.4% of the theoretical values. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). Compounds **8a–d**[15–21], **9a–c**[22–25] were prepared according to the reported procedures.

General method for preparation of diarylpyrazoles (10a–l)

A solution of the appropriate enaminone (**8a–d**, 0.1 mol) in ethanol (50 mL) was heated under reflux with a mixture of *p*-substituted-phenylhydrazine hydrochloride (**9a–c**, 0.1 mol) for 36 h, cooled and diluted with cold water. The precipitated crude product was filtered and recrystallized from ethanol to give the respective pyrazoles (**10a–l**). Physical and spectral data are listed below.

*4-[5-(4-Bromophenyl)-pyrazol-1-yl]benzenesulfonamide (**10a**)*

68% yield; white solid; m.p. 269–271 °C; IR (KBr disk) 3375, 3202 (NH_2), 1336, 1162 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.55 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.12 (d, $J = 8.0$ Hz, 2H, bromophenyl H-3, H-5), 7.28 (s, 2H, NH_2, D_2O exchangeable) 7.45 (d, $J = 8.2$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.51 (d, $J = 8.0$ Hz, 2H, bromophenyl H-2, H-6), 7.78 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.91 (d, $J = 8.2$ Hz, 2H, aminosulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 109.33, 123.24, 124.57, 124.99, 127.54, 127.62, 128.96, 130.30, 131.25, 132.14, 143.17; MS (*m/z*, relative abundance %): 378.24 ($M^{+\cdot}$, 1.17); Anal. Calcd for $C_{15}H_{12}BrN_3O_2S$: C, 47.63; H, 3.20; N, 11.11; Found: C, 47.35; H, 3.35; N, 11.35.

*5-(4-Bromophenyl)-1-(4-methanesulfonyl-phenyl)-1*H*-pyrazole (**10b**)*

72% yield; white solid; m.p. 241–243 °C; IR (KBr disk) 1308, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.05 (s, 1H, $SO_2\underline{CH_3}$), 6.56 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.12 (d, $J = 8.0$ Hz, 2H, bromophenyl H-3, H-5), 7.51 (m, 4H, methanesulfonylphenyl H-3, H-5 and bromophenyl H-3, H-5), 7.79 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.93 (d, $J = 8.2$ Hz, 2H, methanesulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 44.49, 109.53, 124.68, 125.11, 128.50, 128.58, 129.43, 130.05, 131.02, 135.16, 140.36, 141.60; MS (*m/z*, relative abundance %): 377.26 ($M^{+\cdot}$, 0.92); Anal. Calcd for $C_{16}H_{13}BrN_2O_2S$: C, 50.94; H, 3.47; N, 7.43; Found: C, 50.59; H, 3.35; N, 7.65.

*4-[5-(4-Bromophenyl)pyrazol-1-yl]benzoic acid (**10c**)*

56% yield; white solid; m.p. 205–207 °C; IR (KBr disk) 3430 (OH), 1683 (C=O) 1381, 1172 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.55 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.19 (d, $J = 7.8$ Hz, 2H, bromophenyl H-3, H-5), 7.35 (d, $J = 7.8$ Hz, 2H, benzoic H-3, H-5), 7.42 (d, $J = 8.0$ Hz, 2H, bromophenyl H-2, H-6), 7.80 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 8.10 (d, $J = 7.8$ Hz, 2H, benzoic H-2, H-6), 13.11 (s, 1H, COOH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 109.07, 124.57, 128.05, 128.72, 128.98, 129.04, 129.80, 130.05, 130.47, 134.86, 141.07, 170.22; MS (*m/z*, relative abundance %): 343.17 ($M^{+\cdot}$, 3.12); Anal. Calcd for $C_{16}H_{11}BrN_2O_2$: C, 56.00; H, 3.23; N, 8.16; Found: C, 56.35; H, 3.35; N, 7.95.

*4-[5-(4-Nitrophenyl)pyrazol-1-yl]benzenesulfonamide (**10d**)*

88% yield; yellow solid; m.p. 255–257 °C; IR (KBr disk) 3423, 3338 (NH_2), 1339, 1165 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.87 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.50 (m, 2H, NH_2, D_2O exchangeable and 2H, nitrophenyl H-3, H-5), 7.54 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.79 (d, $J = 8.2$ Hz, 2H, nitrophenyl H-2, H-6), 7.92 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 8.24 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 110.78, 124.40, 125.77, 127.37, 130.24, 136.53, 141.27, 141.85, 142.06, 143.55, 147.56; MS (*m/z*, relative abundance %): 344.35 ($M^{+\cdot}$, 9.34); Anal. Calcd for $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27; Found: C, 52.44; H, 3.35; N, 15.95.

*1-(4-Methanesulfonylphenyl)-5-(4-nitrophenyl)-1*H*-pyrazole (**10e**)*

74% yield; yellow solid; m.p. 222–224 °C; IR (KBr disk) 1301, 1149 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 1H, $SO_2\underline{CH_3}$), 6.59 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.44 (d, $J = 8.2$ Hz, 2H, nitrophenyl H-3, H-5), 7.50 (d, $J = 8.2$ Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.84 (d, $J = 1.6$ Hz,1H, pyrazole H-4), 7.96 (d, $J = 8.2$ Hz, 2H, nitrophenyl H-3, H-5), 8.24 (d, $J = 8.2$ Hz, 2H, methanesulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 44.50, 110.57, 124.19, 125.31, 128.74, 129.48, 136.19, 139.54, 141.08, 141.87, 143.68, 144.14; MS (*m/z*, relative abundance %): 343.36 ($M^{+\cdot}$, 35.57); Anal. Calcd for $C_{16}H_{13}N_3O_4S$: C, 55.97; H, 3.82; N, 12.24; Found: C, 55.61; H, 3.45; N, 11.95.

*4-[5-(4-Nitrophenyl)pyrazol-1-yl]benzoic acid (**10f**)*

69% yield; yellowish white solid; m.p. 256–258 °C; IR (KBr disk) 3426 (OH), 1685 (C=O) 1413, 1152 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.92 (d, $J = 1.6$ Hz, 1H pyrazole H-3), 7.41 (d, $J = 7.8$ Hz, 2H, nitrophenyl H-3, H-5), 7.52 (d, $J = 7.8$ Hz, 2H, benzoic H-3, H-5), 7.91 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.98 (d, $J = 7.8$ Hz, 2H, bromophenyl H-2, H-6), 8.12 (d, $J = 7.8$ Hz, 2H, benzoic H-2, H-6), 13.13 (s, 1H, COOH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 110.66, 124.36, 125.44, 125.52, 129.42, 130.14, 130.39, 130.77, 130.94, 136.54, 141.22, 167.00; MS (*m/z*, relative abundance %): 309.01 ($M^{+\cdot}$, 6.22); Anal. Calcd for $C_{16}H_{11}N_3O_4$: C, 62.14; H, 3.58; N, 13.59; Found: C, 62.35; H, 3.35; N, 13.95.

*4-[5-(4-Chlorophenyl)pyrazol-1-yl]benzenesulfonamide (**10g**)*

62% yield; white solid; m.p. 229–231 °C; IR (KBr disk) 3346, 3194 (NH_2), 1338, 1162 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.55 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.19 (d, $J = 8.2$ Hz, 2H,

chlorophenyl H-3, H-5), 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.32 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.41 (d, $J = 8.2$ Hz, 2H, chlorophenyl H-2, H-6), 7.79 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 8.09 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 109.34, 124.56, 125.01, 127.55, 127.62, 129.20, 129.37, 130.06, 131.04, 140.18, 141.39; MS (*m/z*, relative abundance %): 333.03 ($M^{+\cdot}$, 70.42); Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59; Found: C, 54.35; H, 3.35; N, 12.75.

*5-(4-Chlorophenyl)-1-(4-methanesulfonylphenyl)-1*H*-pyrazole (**10h**)*

79% yield; yellow solid; m.p. 236–238 °C; IR (KBr disk) 1305, 1150 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 1H, $SO_2\underline{CH_3}$), 6.56 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 7.19 (d, $J = 7.8$ Hz, 2H, chlorophenyl H-3, H-5), 7.36 (d, $J = 7.8$ Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.51 (d, $J = 7.8$ Hz, 2H, chlorophenyl H-3, H-5), 7.79 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.93 (d, $J = 7.8$ Hz, 2H, methanesulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 44.53, 109.52, 123.32, 125.11, 128.50, 128.91, 130.29, 132.19, 138.94, 141.66, 142.32, 144.02; MS (*m/z*, relative abundance %): 332.04 ($M^{+\cdot}$, 71.47); Anal. Calcd for $C_{16}H_{13}ClN_2O_2S$: C, 57.74; H, 3.94; N, 8.42; Found: C, 57.39; H, 3.75; N, 8.15.

*4-[5-(4-Chlorophenyl)pyrazol-1-yl]benzoic acid (**10i**)*

77% yield; yellowish white solid; m.p. 244–246 °C; IR (KBr disk) 3431 (OH), 1684 (C=O) 1381, 1174 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.55 (d, $J = 1.6$ Hz, 1H pyrazole H-3), 7.18 (d, $J = 8.0$ Hz, 2H, chlorophenyl H-3, H-5), 7.35 (d, $J = 7.8$ Hz, 2H, benzoic H-3, H-5), 7.45 (d, $J = 8.0$ Hz, 2H, chlorophenyl H-2, H-6), 7.78 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.91 (d, $J = 8.0$ Hz, 2H, benzoic H-2, H-6), 13.11 (s, 1H, COOH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 109.08, 124.53, 124.57, 127.90, 128.71, 128.98, 129.04, 130.05, 134.87, 141.04, 143.94, 169.38; MS (*m/z*, relative abundance %): 298.06 ($M^{+\cdot}$, 100.00); Anal. Calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38; Found: C, 64.35; H, 3.35; N, 8.95.

*4-(5-Thiophen-2-yl-pyrazol-1-yl)benzenesulfonamide (**10j**)*

77% yield; brown solid; m.p. 258–260 °C; IR (KBr disk) 3427, 3302 (NH_2), 1341, 1160 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.61 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 6.90 (s, 1H, thienyl H-4), 7.03 (s, 1H, thienyl H-5), 7.28 (s, 2H, NH_2, D_2O exchangeable), 7.38 (d, $J = 4.4$ Hz, 1H, thienyl H-3), 7.56 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.76 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 7.95 (d, $J = 8.0$ Hz, 2H, aminosulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 109.71, 125.49, 127.37, 127.42, 127.71, 128.11, 141.28, 141.63, 145.00, 145.22, 145.41; MS (*m/z*, relative abundance %): 305.07 ($M^{+\cdot}$, 6.32); Anal. Calcd for $C_{13}H_{11}N_3O_2S_2$: C, 51.13; H, 3.63; N, 13.76; Found: C, 51.35; H, 3.35; N, 13.95.

*1-(4-Methanesulfonylphenyl)-5-thiophen-2-yl-1*H*-pyrazole (**10k**)*

58% yield; orange solid; m.p. 233–235 °C; IR (KBr disk) 1384, 1152 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.07 (s, 3H, $SO_2\underline{CH_3}$), 6.61 (d, $J = 1.2$ Hz, 1H, pyrazole H-3), 6.89 (d, $J = 3.6$ Hz, thienyl H-4), 7.02 (dd, $J = 4.0$, 8.8 Hz, thienyl H-4), 7.39 (d, $J = 5.2$ Hz, 1H, thienyl H-5), 7.60 (d, $J = 8.4$ Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.76 (d, $J = 1.2$ Hz, 1H, pyrazole H-4), 7.96 (d, $J = 8.4$ Hz, 2H, methanesulfonylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 44.56, 109.91, 125.10, 125.61, 127.53, 127.77, 128.22, 129.00, 130.11, 130.36, 141.38, 143.97; MS (*m/z*, relative abundance %): 304.09 ($M^{+\cdot}$, 21.83); Anal. Calcd for $C_{14}H_{12}N_2O_2S_2$: C, 55.24; H, 3.97; N, 9.20; Found: C, 55.00; H, 3.71; N, 8.88.

*4-(5-Thiophen-2-yl-pyrazol-1-yl)benzoic acid (**10l**)*

72% yield; brown solid; m.p. 297–299 °C; IR (KBr disk) 3428 (OH), 1693 (C=O) 1347, 1173 (SO_2); ^{1}H NMR (DMSO-d_6) δ 6.67 (d, $J = 1.6$ Hz, 1H, pyrazole H-3), 6.02 (s, 1H, thienyl H-4), 7.07 (s, 1H, thienyl H-5), 7.48 (d, $J = 7.6$ Hz, 2H, benzoic H-3, H-5), 7.62 (d, $J = 4.4$ Hz, 1H, thienyl H-3), 7.81 (d, $J = 1.6$ Hz, 1H, pyrazole H-4), 8.05 (d, $J = 7.6$ Hz, 2H, benzoic H-2, H-6), 13.19 (s, 1H, COOH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 109.71, 125.49, 127.37, 127.42, 127.71, 128.11, 141.05, 141.28, 141.63, 145.00, 145.22, 169.81; MS (*m/z*, relative abundance %): 270.05 ($M^{+\cdot}$, 3.18); Anal. Calcd for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36; Found: C, 62.35; H, 3.35; N, 9.95.

Biological evaluation

COX-1/COX-2 inhibition colorimetric assay

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI) according to the previously reported method[26].

In vivo anti-inflammatory activity

Animals

Adult male Wistar Albino rats (100–150 g) were used in the pharmacological studies. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27 ± 2 °C), with access to food and water. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.

The dose causing 50% edema inhibition (ED_{50}) for test compounds **10a–l** and reference drugs celecoxib and ibuprofen were determined using the *in vivo* carrageenan-induced rat paw edema model and the measurement of paw thickness was done at 3 h after oral administration of the test compound as reported previously[27].

Ulcerogenic liability study

Ulcerogenic liability of the target compounds **10a–l** were determined using ED_{50} dose in comparison with celecoxib (ED_{50} dose) and small dose of Ibuprofen (120 μmol/kg) according to the previously reported method[29].

Declaration of interest

The authors have declared no conflict of interest.

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