Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: http://www.tandfonline.com/loi/ienz20

Synthesis, cyclooxygenase inhibition and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1H-pyrazole derivatives possessing methanesulphonyl pharmacophore

Khaled R. A. Abdellatif, Mohammed T. Elsaady, Salah A. Abdel-Aziz & Ahmed H. A. Abusabaa

To cite this article: Khaled R. A. Abdellatif, Mohammed T. Elsaady, Salah A. Abdel-Aziz & Ahmed H. A. Abusabaa (2016): Synthesis, cyclooxygenase inhibition and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1H-pyrazole derivatives possessing methanesulphonyl pharmacophore, Journal of Enzyme Inhibition and Medicinal Chemistry, DOI: 10.3109/14756366.2016.1158168

To link to this article: http://dx.doi.org/10.3109/14756366.2016.1158168

Published online: 12 Apr 2016.

Journal of Enzyme Inhibition and Medicinal Chemistry

http://informahealthcare.com/enz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, Early Online: 1–11
 DOI: 10.3109/14756366.2016.1158168

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Synthesis, cyclooxygenase inhibition and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1*H*-pyrazole derivatives possessing methanesulphonyl pharmacophore

Khaled R. A. Abdellatif[1], Mohammed T. Elsaady[2], Salah A. Abdel-Aziz[3], and Ahmed H. A. Abusabaa[3]

[1]*Department of Pharmaceutical Organic Chemistry, Beni-Suef University, Beni-Suef, Egypt,* [2]*Department of Medicinal Chemistry, Beni-Suef University, Beni-Suef, Egypt, and* [3]*Department of Pharmaceutical Medicinal Chemistry, Al-Azhar University, Assuit, Egypt*

Abstract

A new series of 1,3,5-triaryl-4,5-dihydro-1*H*-pyrazole derivatives **13a**–**p** were synthesized *via* aldol condensation of 3/4-nitroacetophenones with appropriately substituted aldehydes followed by cyclization of the formed chalcones with 4-methanesulfonylphenylhydrazine hydrochloride. All the synthesized compounds were evaluated for their cyclooxygenase (COX) inhibition, anti-inflammatory activity and ulcerogenic liability. All compounds were more potent inhibitors for COX-2 than COX-1. While most compounds showed good anti-inflammatory activity, compounds **13d**, **13f**, **13k** and **13o** were the most potent derivatives ($ED_{50} = 66.5$, 73.4, 79.8 and 70.5 μmol/kg, respectively) in comparison with celecoxib ($ED_{50} = 68.1$ μmol/kg). Compounds **13d**, **13f**, **13k** and **13o** (ulcer index = 3.89, 4.86, 4.96 and 3.92, respectively) were 4–6 folds less ulcerogenic than aspirin (ulcer index = 22.75) and showed approximately ulceration effect similar to celecoxib (ulcer index = 3.35). In addition, molecular docking studies were performed for compounds **13d**, **13f**, **13k** and **13o** inside COX-2 active site which showed acceptable binding interactions (affinity in kcal/mol −2.1774, −6.9498) in comparison with celecoxib (affinity in kcal/mol −6.5330).

Keywords

Anti-inflammatory, cyclooxygenase inhibition, dihydropyrazole

History

Received 1 November 2015
Revised 7 February 2016
Accepted 9 February 2016
Published online 12 April 2016

Introduction

Traditional non-steroidal anti-inflammatory drugs (NSAIDs) reduce the pain and inflammation through inhibition of both cyclooxygenase-1 and -2 (COX-1 and COX-2)[1]. While COX-1 enzyme is constitutively expressed and plays an important role as a housekeeping enzyme such as protection of gastric mucosa, vascular homeostasis and platelet aggregation, COX-2 enzyme is expressed and significantly upregulated during acute and chronic inflammation, pain and oncogenesis[2]. Non-selective NSAIDs such as aspirin, ibuprofen and indomethacin inhibit the activities of both enzymes and in turn inhibit the production of gastro protective prostaglandins (PGs) synthesized through COX-1 pathway causing gastro intestinal side effects like ulcers, perforation and bleeding[3]. For this reason, selective COX-2 inhibitor drugs (coxibs) such as celecoxib (**1**), rofecoxib (**2**) and valdecoxib (**3**) (Figure 1) which have the same anti-inflammatory efficacy as traditional NSAIDs with minimized unwanted gastrointestinal side effects take much consideration in the last two decades[4]. Unfortunately, highly selective COX-2 inhibitors caused cardiovascular side effects such as increased systemic blood pressure and myocardial infarction that ultimately prompted the withdrawal of rofecoxib and valdecoxib[5]. At present, celecoxib (**1**) is the only and highly marketed coxib, so, there is still a need for synthesis of COX-2 inhibitor with no adverse effects (Figure 2). We have previously reported[6–11] some derivatives of celecoxib (**4**–**8**) which showed considerable anti-inflammatory activity.

In continuation of our previous work for development of safe anti-inflammatory derivatives, we now describe the synthesis, *in vitro* evaluation as COX-1/COX-2 inhibitors, *in vivo* anti-inflammatory (AI) activity and ulcerogenic liability for a new series of 1,3,5-triaryl-4,5-dihydro-1*H*-pyrazoles **13a**–**p** as celecoxib derivatives in which; (i) pyrazole ring of celecoxib was replaced with 4,5-dihydropyrazole nucleus, (ii) trifluoromethyl moiety was replaced with substituted aryl moiety; it was reported that the substituent at pyrazole C-3 has little steric restrictions for COX-2, and observed that COX-2 inhibition should be remained[12], (iii) tolyl group was maintained or replaced with different substituted aryl or heteroaryl moieties and (iv) the COX-2 pharmacophore (sulphamoyl, SO_2NH_2) group on the N^1-phenyl ring was replaced with another COX-2 pharmacophoric moiety (methanesulfonyl, SO_2CH_3).

Experimental

Chemistry

Melting points were determined on a Griffin apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu 435 spectrometer using KBr discs. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz spectrometer (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt) in D_2O,

Address for correspondence: Khaled R. A. Abdellatif, Ph.D., Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt. Tel: +002 0100 2535444. Fax: +002 082 2317958. E-mail: khaled.ahmed@pharm.bsu.edu.eg

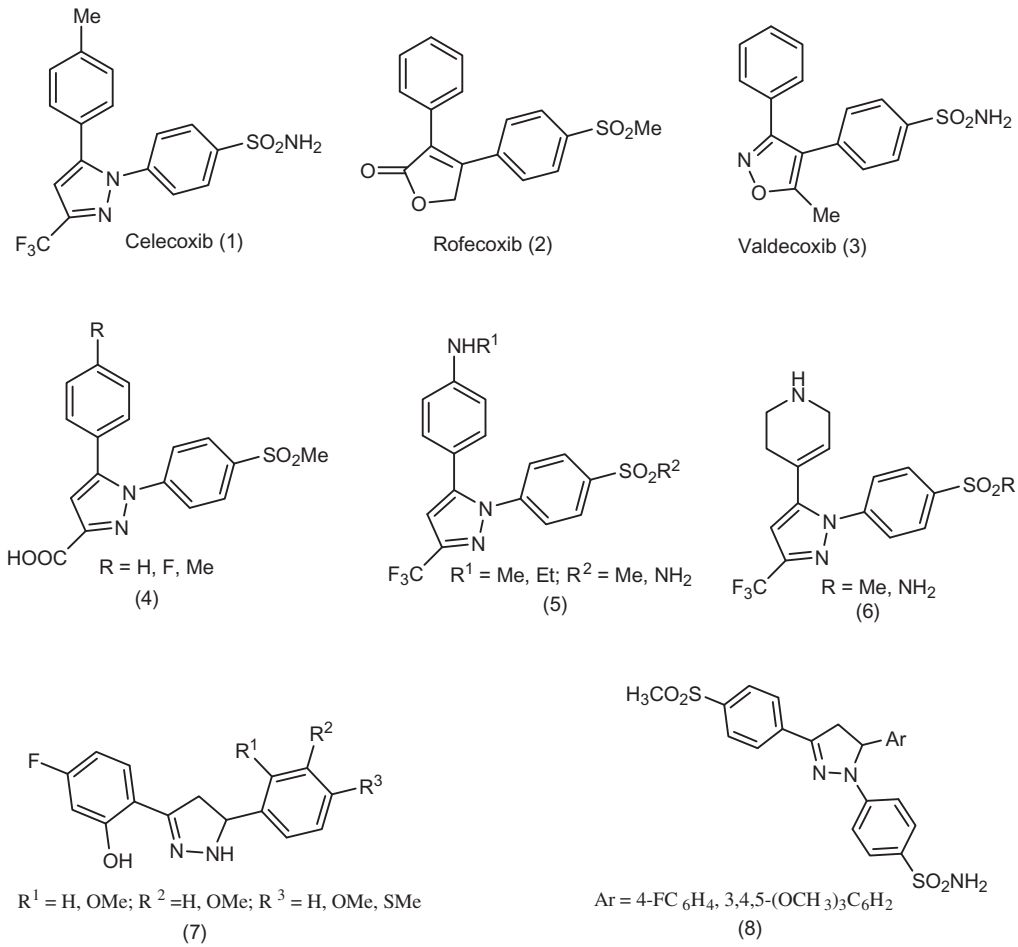
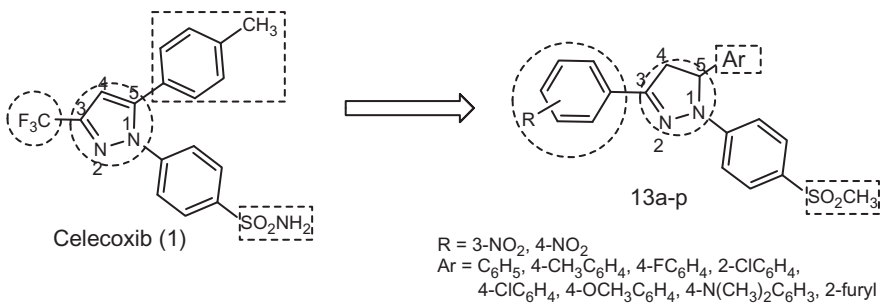


Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**1**), rofecoxib (**2**) and valdecoxib (**3**), and celecoxib analogs (**4**–**8**).

Figure 2. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitor celecoxib (**1**) and the designed 1,3,5-triaryldihydropyrazoles **13a**–**p**.



DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values were estimated in hertz (Hz). Mass spectra were run on Hewlett Packard 5988 spectrometer. Microanalysis was performed for C, H, N at the Micro Analytical Center, Cairo University, Egypt and was within ±0.4% of theoretical values. All other reagents, purchased from the Acros Chemical Company (Milwaukee, WI), were used without further purification. Chalcones **11a**[13], **11b–d**[14], **11e**[15], **11f**[14] **11 g**[16], **11 h**[17], **11i–j**[18], **11k**[19], **11l**[20], **11m**[21], **11n**[18], **11o**[22], **11p**[23] and 4-methanesulfonylphenylhydrazine hydrochloride (**12**)[24] were prepared according to reported procedures.

Spectral data (14) for chalcones 11b, 11c, 11d and 11f are listed below

*3-(4-Chlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**11b**)*

IR (KBr disk) 3097(CH), 1662 (C=O), 1606 (Ar–C=C); ^{1}H NMR (DMSO-d_6) δ 7.57–7.54 (m, 2H, Ar–H), 7.83–7.77 (d, 1H, CH=CH, $J = 15.5$ Hz), 8.02–7.95 (m, 3H, Ar–H and CH=CH), 8.38 (s, 4H, Ar–H).

*3-(4-Fluorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**11c**)*

IR (KBr disk) 1609 (Ar–C=C), ^{1}H NMR (DMSO-d_6) 7.36–7.30 (m, 2H, Ar–H), 7.83 (s, 1H, CH=CH), 7.90 (s, 1H, CH=CH), 8.03–8.00 (m, 2H, Ar–H), 8.40–8.33 (m, 4H, Ar–H).

*3-(4-(Dimethylamino) phenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**11d**)*

IR (KBr disk) 3047 (C–H), 2909 (C–H), 1653 (C=O), 1598 (Ar–C=C), 3.02 (s, 6H, $N(CH_3)_2$, 6.77–6.74 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.64–7.60 (d, 1H, CH=CH, $J = 11.7$ Hz), 7.75–7.71 (m, 3H, Ar–H and CH=CH), 8.36–8.28 (m, 4H, Ar–H).

*3-(4-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**11f**)*

IR (KBr disk) 1655 (C=O), 1595 (Ar–C=C); ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H, OCH_3), 7.03 (d, 2H, Ar–H, $J = 6$ Hz),7.77 (d, 2H, Ar–H, $J = 12$ Hz), 7.86 (d, 2H, CH=CH, $J = 16.5$ Hz), 8.38–8.29 (m, 4H, Ar–H).

General procedure for preparation of 1,3,5-triaryl-4,5-dihydro-*1H*-pyrazoles (13a–p)

To a solution of the appropriate chalcone **11a**–**p** (2.0 mmol) in ethanol (20 mL), 4-methanesulfonylphenylhydrazine hydrochloride (**12**, 3.0 mmol, 0.666 g) was added and the reaction mixture was heated under reflux for 12–18 h. The reaction was monitored every 60 min interval on TLC plates using chloroform/methanol (9.5:0.5 V/V). After completion of the reaction, the mixture was poured into ice cold water. The obtained solid was filtered, washed with water, dried and crystallized from ethyl acetate to give the respective triarylpyrazoles **13a**–**p**. Physical and spectral data for **13a**–**p** are listed below.

*1-(4-Methanesulfonylphenyl)-3-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole (**13a**)*

Yield: 58%; orange powder; m.p. 118–120 °C; IR (KBr disk) 2923 (C–H aliphatic), 1322, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, SO_2CH_3), 3.29 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.06 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.79 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.22 (d, $J = 8.8$ Hz, 2H, phenyl H-3, H-5), 7.27–7.30 (m, 3H, phenyl H-2, H-6, H-4), 7.37 (d, $J = 7.2$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.75 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.96 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.30 (d, $J = 9.2$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 43.15 (pyrazole C-4), 44.88 (SO_2CH_3), 64.12 (pyrazole C-5), 113.28, 124.08, 125.51, 126.52, 128.44, 128.97, 129.74, 130.34, 137.97, 140.39, 147.08, 147.19, 147.75; MS (*m*/*z*): 421 ($M^{+\cdot}$, 5.10%); Anal. Calcd. for $C_{22}H_{19}N_3O_4S$: C, 62.69; H, 4.54; N, 9.97. Found: C, 62.81; H, 4.60; N, 10.12.

*5-(4-Chlorophenyl)-1-(4-methanesulfonylphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13b**)*

Yield: 63%; orange powder; m.p. 264–266 °C; IR (KBr disk) 2924 (C–H aliphatic), 1331, 1137 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, SO_2CH_3), 3.30 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.05 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.81 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.21 (d, $J = 8.8$ Hz, 2H, chlorophenyl H-2, H-6), 7.32 (d, $J = 8.4$ Hz, 2H, chlorophenyl H-3, H-5), 7.44 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.72 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.03 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-2, H-6), 8.30 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 42.86 (pyrazole C-4), 44.51 (SO_2CH_3), 62.69 (pyrazole C-5), 113.31, 124.46, 127.54, 128.26, 129.15, 129.71, 130.90, 132.89, 138.26, 140.49, 146.77, 147.72, 149.04; MS (*m*/*z*): 455 ($M^{+\cdot}$, 77.15%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SCl$: C, 57.96; H, 3.98; N, 9.22. Found: C, 57.9; H, 3.93; N, 9.35.

*5-(4-Flourophenyl)-1-(4-methanesulfonylphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13c**)*

Yield: 71%; orange powder; m.p. 226–228 °C; IR (KBr disk) 2927 (C–H aliphatic), 1336, 1140 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, SO_2CH_3), 3.29 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.03 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.82 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.17–7.21 (m, 2H, flourophenyl H-2, H-6), 7.21 (d, $J = 6.8$ Hz, 2H, flourophenyl H-3, H-5), 7.33 (d, $J = 5.2$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.72 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.03 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.29 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 43.18 (pyrazole C-4), 44.86 (SO_2CH_3), 63.48 (pyrazole C-5), 113.3, 116.75, 124.10, 126.54, 127.35, 129.00, 130.66, 136.19, 137.82, 146.96, 147.15, 147.86, 162.62 (d, $J = 247$ Hz, *C*-F); MS (*m*/*z*): 439 ($M^{+\cdot}$, 99.21%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SF$: C, 60.13; H, 4.13; N, 9.56. Found: C, 60.41; H, 4.17; N, 9.64.

*5-(4-Dimethylaminophenyl)-1-(4-methanesulfonylphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13d**)*

Yield: 60%; red crystals; m.p. 162–165 °C; IR (KBr disk) 2918 (C–H aliphatic), 1334, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.96 (s, 6H, $N(CH_3)_2$), 3.10 (s, 3H, SO_2CH_3), 3.26 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.04 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.77 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.11 (d, $J = 8.4$ Hz, 2H, dimethylaminophenyl H-3, H-5), 7.23 (d, $J = 8.8$ Hz, 2H, dimethylaminophenyl H-2, H-6), 7.72 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.81 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.04 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.30 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 43.13 (pyrazole C-4), 44.89 (SO_2CH_3), 50.88 ($N(CH_3)_2$), 63.8 (pyrazole C-5), 113.30, 124.06, 124.57, 126.44, 126.62, 128.90, 129.03, 129.29, 129.73, 130.04, 138.20, 147.22, 147.65; MS (*m*/*z*): 464 ($M^{+\cdot}$, 42.01%); Anal. Calcd. for $C_{24}H_{24}N_4O_4S$: C, 62.05; H, 5.21; N, 12.06. Found: C, 62.17; H, 5.29; N, 12.31.

*1-(4-Methanesulfonylphenyl)-5-(4-methylphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13e**)*

Yield: 45%; orange powder; m.p. 214–216 °C; IR (KBr disk) 2976 (C–H aliphatic), 1328, 1135 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 3.09 (s, 3H, SO_2CH_3), 3.25 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.03 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.74 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.15 (s, 4H, methylphenyl H-2, H-6, H-3, H-5), 7.22 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.71 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.04 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.30 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 21.10 (CH_3), 43.03 (pyrazole C-4), 44.50 (SO_2CH_3), 63.18 (pyrazole C-5), 113.29, 124.48, 126.14, 127.46, 129.06, 130.26, 130.61, 137.63, 138.40, 138.60, 146.89, 147.62, 148.93; MS (*m*/*z*): 435 ($M^{+\cdot}$, 72.10%); Anal. Calcd. for $C_{23}H_{21}N_3O_4S$: C, 63.43; H, 4.86; N, 9.65. Found: C, 63.60; H, 4.94; N, 9.78.

*1-(4-Methanesulfonylphenyl)-5-(4-methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13f**)*

Yield: 55%; orange powder; m.p. 212–214 °C; IR (KBr disk) 2921 (C–H aliphatic), 1332, 1138 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.00 (s, 3H, SO_2CH_3), 3.79 (s, 3H, OCH_3), 3.23 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 3.93 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.46 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 6.89 (d, $J = 8.0$ Hz, 2H, methoxyphenyl H-2, H-6), 7.18 (d, $J = 7.6$ Hz, 4H, methoxyphenyl H-3, H-5 and methanesulphonylphenyl H-2, H-6), 7.71 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.88 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-2, H-6), 8.24 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 43.19 (pyrazole C-4), 44.89 (SO_2CH_3), 55.34 (OCH_3), 63.69 (pyrazole C-5), 113.31, 114.92, 124.07, 126.49, 126.80, 128.91, 130.26, 132.39, 138.09, 147.13, 147.22, 147.71, 159.55; MS (*m*/*z*): 451 ($M^{+\cdot}$, 100%); Anal. Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.18; H, 4.69; N, 9.31. Found: C, 61.42; H, 4.78; N, 9.47.

*5-(2-Chlorophenyl)-1-(4-methanesulfonyl-phenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13g**)*

Yield: 71%; orange powder; m.p. 220–222 °C; IR (KBr disk) 2918 (C–H aliphatic), 1339, 1139 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.11 (s, 3H, SO_2CH_3), 3.28 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.14 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.94 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.01–7.13 (m, 3H, chlorophenyl H-4, H-5, H-6), 7.37 (d, $J = 6.8$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.60 (s, 1H, chlorophenyl H-3), 7.75 (d, $J = 9.2$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.04 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.29 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 41.77 (pyrazole C-4), 44.87 (SO_2CH_3), 61.06 (pyrazole C-5), 113.18, 124.08, 126.59, 127.93, 129.11, 129.66, 130.44, 130.67, 131.78, 137.05, 137.80, 146.81, 147.69, 147.86; MS (*m/z*): 455 ($M^{+\cdot}$, 24.27%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SCl$: C, 57.96; H, 3.98; N, 9.22. Found: C, 57.92; H, 4.05; N, 9.40.

*5-(2-Furyl)-1-(4-methanesulfonyl-phenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13h**)*

Yield: 62%; grey powder; m.p. 152–154 °C; IR (KBr disk) 2924 (C–H aliphatic), 1334, 1137 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.12 (s, 3H, SO_2CH_3), 3.52 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 3.91 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.93 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 6.41 (s, 1H, furyl H-5), 6.58 (s, 1H, furyl H-4), 7.37 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.58 (s, 1H, furyl H-3), 7.75 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 8.05 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-2, H-6), 8.31 (d, $J = 8.8$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 39.72 (pyrazole C-4), 44.88 (SO_2CH_3), 57.52 (pyrazole C-5), 110.64, 113.14, 120.80, 124.08, 126.55, 128.96, 129.76, 130.35, 131.54, 133.60, 147.40, 148.64, 151.53; MS (*m/z*): 411 ($M^{+\cdot}$, 73.78%); Anal. Calcd. for $C_{20}H_{17}N_3O_5S$: C, 58.38; H, 4.16; N, 10.21. Found: C, 58.52; H, 4.23; N, 10.37.

*1-(4-Methanesulfonyl-phenyl)-3-(3-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole (**13i**)*

Yield: 65%; yellow powder; m.p. 194–196 °C; IR (KBr disk) 2922 (C–H aliphatic), 1349, 1132 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, SO_2CH_3), 3.30 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.08 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.76 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.20 (d, $J = 8.8$ Hz, 2H, phenyl H-3, H-5), 7.25–7.34 (m, 3H, phenyl H-2, H-6, H-4), 7.37 (d, $J = 6.8$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.70 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.75 (t, $J = 7.6$ Hz, 1H, nitrophenyl H-5), 8.21 (d, $J = 7.6$ Hz, 1H, nitrophenyl H-6), 8.26 (d, $J = 6$ Hz, 1H, nitrophenyl H-4), 8.55 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 43.35 (pyrazole C-4), 44.91 (SO_2CH_3), 63.93 (pyrazole C-5), 113.08, 120.78, 123.70, 125.51, 128.39, 128.98, 129.60, 129.74, 130.34, 131.51, 133.73, 140.39, 147.26; MS (*m/z*): 421 ($M^{+\cdot}$, 100%); Anal. Calcd. for $C_{22}H_{19}N_3O_4S$: C, 62.69; H, 4.54; N, 9.97. Found: C, 62.88; H, 4.58; N; 10.04.

*5-(4-Chlorophenyl)-1-(4-methanesulfonyl-phenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13j**)*

Yield: 45%; yellow powder; m.p. 222–224 °C; IR (KBr disk) 2922 (C–H aliphatic), 1347, 1132 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, SO_2CH_3), 3.37 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.06 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.79 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.19 (d, $J = 8.8$ Hz, 2H, chlorophenyl H-2, H-6), 7.32 (d, $J = 8.8$ Hz, 2H, chlorophenyl H-3, H-5), 7.44 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.72 (d, $J = 9.2$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.77 (t, $J = 8.0$ 1H, nitrophenyl H-5), 8.20 (d, $J = 8.0$ Hz, 1H, nitrophenyl H-6), 8.27 (d, $J = 8$ Hz, 1H, nitrophenyl H-4), 8.54 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 43.03 (pyrazole C-4), 44.56 (SO_2CH_3), 62.47 (pyrazole C-5), 113.07, 120.72, 124.17, 128.28, 129.15, 129.66, 130.49, 130.91, 132.83, 133.69, 140.56, 147.01, 148.65, 149.25; MS (*m/z*): 455 ($M^{+\cdot}$, 24.53%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SCl$: C, 57.96; H, 3.98; N, 9.22. Found: C, 57.85; H, 3.86; N, 9.22.

*5-(4-Flourophenyl)-1-(4-methanesulfonyl-phenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13k**)*

Yield: 65%; yellow powder; m.p. 192–194 °C; IR (KBr disk) 2923 (C–H aliphatic), 1347, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, SO_2CH_3), 3.29 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.06 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.78 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.16–7.20 (m, 2H, flourophenyl H-2, H-6), 7.20 (d, $J = 3.2$ Hz, 2H, flourophenyl H-3, H-5), 7.33 (d, $J = 3.2$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.71 (d, $J = 9.2$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.77 (t, $J = 8$ Hz, 1H, nitrophenyl H-5), 8.20 (d, $J = 8$ Hz, 1H, nitrophenyl H-6), 8.26 (d, $J = 6.4$ Hz, 1H, nitrophenyl H-4), 8.54 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 43.36 (pyrazole C-4), 44.89 (SO_2CH_3), 63.25 (pyrazole C-5), 113.09, 116.70, 120.78, 123.79, 127.35, 128.99, 129.79, 130.23, 131.52, 133.60, 136.27, 147.26, 148.63, 162.47 (d, $J = 247$ Hz, *C*-F); MS (*m/z*): 439 ($M^{+\cdot}$, 100%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SF$: C, 60.13; H, 4.13; N, 9.56. Found: C, 59.92; H, 3.89; N, 9.66.

*5-(4-Dimethylaminophenyl)-1-(4-methanesulfonyl-phenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13l**)*

Yield: 47%; green powder; m.p. 192–194 °C; IR (KBr disk) 2917 (C–H aliphatic), 1349, 1141 (SO_2); 1H NMR (DMSO-d_6) δ 2.84 (s, 6H, $N(CH_3)_2$), 3.08 (s, 3H, SO_2CH_3), 3.25 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.00 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.62 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 6.68 (d, $J = 8.8$ Hz, 2H, dimethylaminophenyl H-3, H-5), 7.10 (d, $J = 8.8$ Hz, 2H, dimethylaminophenyl H-2, H-6), 7.22 (d, $J = 9.2$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.69 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.77 (t, $J = 8.0$ Hz, 1H, nitrophenyl H-5), 8.19 (d, $J = 7.6$ Hz, 1H, nitrophenyl H-6), 8.25 (d, $J = 6.0$ Hz, 1H, nitrophenyl H-4), 8.55 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 43.61 (pyrazole C-4), 44.92 ($SO2CH_3$), 50.88 ($N(CH_3)_2$), 63.57 (pyrazole C-5), 113.09, 113.19, 120.71, 123.55, 126.64, 128.90, 129.02, 129.71, 131.47, 133.93, 147.33, 147.43, 148.61; MS (*m/z*): 464 (M+., 36.40%); Anal. Calcd. for $C_{24}H_{24}N_4O_4S$: C, 62.05; H, 5.21; N, 12.06. Found: C, 62.31; H, 5.32; N, 12.17.

*1-(4-Methanesulfonylphenyl)-5-(4-methylphenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13m**)*

Yield: 48%; yellow powder; m.p. 172–174 °C; IR (KBr disk) 2926 (C–H aliphatic), 1348, 1137 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 3.07 (s, 3H, SO_2CH_3), 3.25 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.03 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.68 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.15 (s, 4H, methylphenyl H-2, H-6, H-3, H-5), 7.22 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.68 (d, $J = 8.4$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.75 (t, $J = 8.0$ Hz, 1H, nitrophenyl H-5), 8.17 (d, $J = 8.0$ Hz, 1H, nitrophenyl H-6), 8.24 (d, $J = 8.0$ Hz, 1H, nitrophenyl H-4), 8.52 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 21.08 (CH_3), 43.19 (pyrazole C-4),

44.52 (SO_2CH_3), 62.96 (pyrazole C-5), 113.04, 120.56, 124.06, 126.14, 129.04, 130.10, 130.22, 130.92, 132.73, 133.76, 137.59, 138.62, 147.14, 148.61, 149.11; MS (*m/z*): 435 ($M^{+\cdot}$, 66.81%); Anal. Calcd. for $C_{23}H_{21}N_3O_4S$: C, 63.43; H, 4.86; N, 9.65. Found: C, 63.3; H, 4.43; N, 9.68.

*1-(4-Methanesulfonylphenyl)-5-(4-methoxyphenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13n**)*

Yield: 84%; yellow powder; m.p. 184–186 °C; IR (KBr disk) 2920 (C–H aliphatic), 1348, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.08 (s, 3H, SO_2CH_3), 3.28 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 3.70 (s, 3H, OCH_3), 4.03 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.69 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 6.92 (d, $J = 8.8$ Hz, 2H, methoxyphenyl H-2, H-6), 7.20 (d, $J = 6.8$ Hz, 2H, methoxyphenyl H-3, H-5), 7.22 (d, $J = 6.0$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.70 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.77 (t, $J = 8$ Hz, 1H, nitrophenyl H-5), 8.20 (d, $J = 8$ Hz, 1H, nitrophenyl H-6), 8.27 (d, $J = 8$ Hz, 1H, nitrophenyl H-4), 8.54 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 43.25 (pyrazole C-4), 44.56 (SO_2CH_3), 55.53(OCH3), 62.73 (pyrazole C-5), 113.08, 115.00, 120.61, 124.06, 127.54, 129.04, 130.21, 130.91, 132.74, 133.53, 133.86, 147.17, 148.66, 149.12, 159.21; MS (*m/z*): 451 ($M^{+\cdot}$, 100%); Anal. Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.18; H, 4.69; N, 9.31. Found: C, 61.44; H, 4.81; N, 9.44.

*5-(2-Chlorophenyl)-1-(4-methanesulfonyl-phenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13o**)*

Yield: 75%; yellow powder; m.p. 198–200 °C; IR (KBr disk) 2926 (C–H aliphatic), 1349, 1139 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, SO_2CH_3), 3.30 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 4.17 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.93 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 7.01–7.13 (m, 3H, chlorophenyl H-4, H-5, H-6), 7.37 (d, J =6.8 Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.60 (s, 1H, chlorophenyl H-3), 7.7 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.75 (s, 1H, nitrophenyl H-5), 8.21 (d, $J = 7.6$ Hz, 1H, nitrophenyl H-4), 8.26 (d, $J = 6.8$ Hz, 1H, nitrophenyl H-6), 8.56 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 41.94 (pyrazole C-4), 44.89 (SO_2CH_3), 61.06 (pyrazole C-5), 112.99, 120.82, 123.83, 126.64, 127.90, 129.12, 129.62, 129.77, 130.32, 130.43, 131.55, 131.81, 133.58, 137.11, 147.03, 147.78, 148.62; MS (*m/z*): 455 ($M^{+\cdot}$, 90.64%); Anal. Calcd. for $C_{22}H_{18}N_3O_4SCl$: C, 57.96; H, 3.98; N, 9.22. Found: C, 57.7; H, 3.80; N, 9.17.

*5-(2-Furyl)-1-(4-methanesulfonylphenyl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole (**13p**)*

Yield: 73%; green powder; m.p. 186–188 °C; IR (KBr disk) 2923 (C–H aliphatic), 1348, 1131 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.11 (s, 3H, SO_2CH_3), 3.56 (dd, $J = 17.6$, 5.6 Hz, 1H, pyrazole H-4), 3.93 (dd, $J = 17.6$, 12.0 Hz, 1H, pyrazole H′-4), 5.91 (dd, $J = 12.0$, 5.6 Hz, 1H, pyrazole H-5), 6.41 (s, 1H, furfural H-5), 6.57 (s, 1H, furfural H-4), 7.37 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-2, H-6), 7.58 (s, 1H, furfural H-3), 7.74 (d, $J = 8.8$ Hz, 2H, methanesulphonylphenyl H-3, H-5), 7.79 (d, $J = 8$ Hz, 1H, nitrophenyl H-5), 8.24 (d, $J = 8$ Hz, 1H, nitrophenyl H-6), 8.28 (d, $J = 8$ Hz, 1H, nitrophenyl H-4), 8.56 (s, 1H, nitrophenyl H-2); ^{13}C NMR (DMSO-d_6) δ 39.92 (pyrazole C-4), 44.92 (SO_2CH_3), 57.36 (pyrazole C-5), 107.90, 110.64, 113.14, 120.80, 123.79, 128.96, 129.76, 130.35, 131.54, 133.60, 142.96, 147.40, 148.64, 151.53; MS (*m/z*): 411 ($M^{+\cdot}$, 79.48%); Anal. Calcd. for $C_{20}H_{17}N_3O_5S$: C, 58.38; H, 4.16; N, 10.21. Found: C, 58.49; H, 4.21; N, 10.42.

Biological evaluation

COX-1/COX-2 inhibition colorimetric assay

The *in vitro* inhibition of ovine COX-1/COX-2 was measured using an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions and as reported before[25].

In vivo anti-inflammatory activity

Animals

Male Wister albino rats with average body weight of 150 g were used in the experiments. The animals were kept under controlled environment of humidity 30–60%, light period of 12 h/day and temperature 27 ± 2 °C with access to food and water. The experimental procedures were carried out in 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.

Carrageenan induced rat paw edema

Rats were divided into 18 groups of five animals each. The first group was administered vehicle; the second one was administrated celecoxib (50 mg/kg) while the remaining groups were administrated test compounds (**13a–p**, 50 mg/kg, and one group per one compound). Immediately thereafter and under light anesthesia, the animals received 100 μL of vehicle or carrageenan (1% in saline) S.C. on the plantar surface of the left hind paw as reported before[26]. The development of paw edema was assessed by measuring paw-volume changes at 1, 3 and 5 h after carrageenan injection using a pair of dial thickness gauge calipers accurate to 0.001 cm. The right hind paw served as a reference of non-inflamed paw for comparison. Results are expressed as paw-volume change (mL).

Additionally, the ED_{50} values for the most potent derivatives (**13d**, **13f**, **13k** and **13o**) were calculated using three different doses and paw thickness of each rat was measured after 3 h of carrageenan injection according to the reported procedure[26].

Ulcerogenic liability study

Ulcerogenic liability for the most biologically active synthesized compounds (**13d**, **13f**, **13k** and **13o**), celecoxib and aspirin was evaluated using the previously reported procedures[27]. Adult male albino rats weighing between 120 and 150 g were used in this study and divided into seven groups each of five animals. The animals were fasted for 20 h before drug administration. The first group was given 1% aqueous solution of tween 80 and was used as the control group. The second and third groups received celecoxib and aspirin, respectively, were used as reference drugs in a dose 100 mg/kg suspended in 1% tween. The remaining groups received the tested compounds (**13d**, **13f**, **13k** and **13o**) in a dose 50 mg/kg suspended in 1% tween. Treatment was continued once daily for three successive days in all groups. Two hours after the last dose, rats were sacrificed under general anesthesia and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10×) for the presence of lesions in the form of hemorrhages or linear breaks and erosions. The expression of the degree of ulcerogenic effect was in terms of dividing the percentage incidence of ulcers in each group of animal by 10, the average number of ulcers per stomach and the visual observation of the ulcer score (average severity of ulcer). The ulcer scores are divided into 0 ulcer score which indicates no ulcer, 1 ulcer score which indicates only mucosal erythema, 2 ulcer score which indicates mild mucosal edema, slight bleeding

or slight erosions, 3 ulcer score which indicates moderate edema, bleeding ulcers or erosions and 4 ulcer index which indicates severe ulceration, erosions, edema and tissue necrosis. The sum of all above values indicates the ulcer index.

Statistical analysis

Significant difference among groups was assessed using two way ANOVA followed by *post hoc* Tukey test. The results were expressed as mean ± standard error (SE). Differences were considered significant at $*p < 0.01$.

Molecular modeling

The crystal structures of celecoxib bound at the COX-2 (PDB ID: 2AW1) active sites[28] (obtained from protein data bank at Research Collaboration for Structural Bioinformatics (RSCB) protein database [PDB]). Docking of the co-crystallized ligand should be carried out to study the scoring energy (*s*), root mean standard deviation (rmsd) and amino acid interactions. Root mean square deviation (rmsd) which is the measure of superposing was 0.50 Å for the lead compounds. Docking was performed using London dG force and refinement of the results was achieved using force field energy. Preparation of the s compounds for docking was achieved *via* their 3D structure built by Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., Montreal, Quebec, Canada). Certain procedures were taken before docking which include: 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligands. Docking for the most active biologically compounds (**13d**, **13f**, **13k** and **13o**) was applied. Binding interactions between the amino acid residues and functional groups of the compounds are summarized in Table 5.

Results and discussion

Chemistry

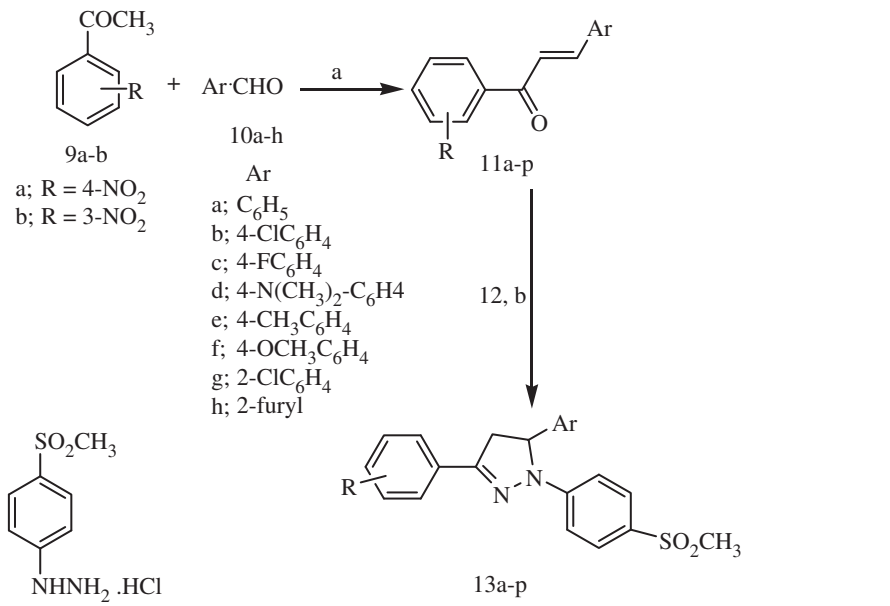
A group of 3-(3/4-nitrophenyl)-5-(substituted-aryl)-1-(4-methanesulphonylphenyl)-4,5-dihydro-1*H*-pyrazoles (**13a–p**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, the reaction of 3-nitro and 4-nitroacetophenone (**9a**, **b**) with different aldehydes **10a–h** provided the corresponding chalcones **11a–p** which upon cyclization with 4-methanesulfonylphenylhydrazine hydrochloride (**12**) in ethanol under reflux conditions gave the target triarylpyrazole derivatives **13a–p** in good yields (45–84%). All the newly synthesized compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses.

The IR spectra of dihydropyrazole compounds **13a–p** showed two sharp peaks at 1141–1131 cm^{-1} and 1349–1322 cm^{-1} corresponding to SO_2. The ^{1}H NMR spectra of dihydropyrazole compounds **13a–p** showed three signals as doublet of doublet (dd), each of one proton intensity, one at δ 3.23–3.56, second at δ 3.91–4.17, and the third at δ 5.46–5.94 with three different *J* values (17.6, 12.0, 5.6 Hz) corresponding to three protons of the dihydropyrazole ring. The highest *J* value was due to geminal coupling of two protons at position 4 while the other two *J* values due to coupling of two geminal protons with proton at position 5. Additionally, ^{13}C NMR spectra of **13a–p** confirmed the presence of dihydropyrazole ring through presence of three peaks at δ 39.72–43.61, 61.06–64.12 and 147.26–149.04 corresponding to dihydropyrazole C-4, C-5 and C-3, respectively.

Biological evaluation

In vitro *COX inhibition assay*

In vitro structure–activity relationships acquired for this group of triarylpyrazole derivatives (**13a–p**) showed that they exhibit a



Scheme 1. Reagents and conditions: (a) MeOH, NaOH, RT, 12 h; (b) EtOH, reflux, 12–18 h.

	R	Ar		R	Ar
a;	4-NO_2	C_6H_5	i;	3-NO_2	C_6H_5
b;	4-NO_2	4-ClC_6H_4	j;	3-NO_2	4-ClC_6H_4
c;	4-NO_2	4-FC_6H_4	k;	3-NO_2	4-FC_6H_4
d;	4-NO_2	4-$N(CH_3)_2C_6H4$	l;	3-NO_2	4-$N(CH_3)_2C_6H4$
e;	4-NO_2	4-$CH_3C_6H_4$	m;	3-NO_2	4-$CH_3C_6H_4$
f;	4-NO_2	4-$OCH_3C_6H_4$	n;	3-NO_2	4-$OCH_3C_6H_4$
g;	4-NO_2	2-ClC_6H_4	o;	3-NO_2	2-ClC_6H_4
h;	4-NO_2	2-furyl	p;	3-NO_2	2-furyl

Table 1. *In vitro* COX-1 and COX-2 inhibition of triarylpyrazoles **13a–p** and celecoxib.

	IC_{50} (μM)[a]		
Compound no.	COX-1	COX-2	COX-2 S.I.[b]
13a	10.4	3.4	3.06
13b	13.4	4.6	2.91
13c	9.6	3.4	2.82
13d	8.7	2.9	3
13e	7.6	1.8	4.22
13f	3.4	0.74	4.59
13g	5.9	1.5	3.93
13h	8.3	1.8	4.61
13i	4.9	1.6	3.06
13j	11.3	5.4	2.09
13k	6.5	2.1	3.09
13l	12.8	4.5	2.84
13m	6.8	1.3	5.23
13n	6.5	1.8	3.61
13o	11.6	4.1	2.83
13p	3.4	0.87	3.91
Celecoxib	7.9	1.3	6.07

[a]IC_{50} value represents the compound concentration that is required to produce 50% inhibition of COX-1 or COX-2 which is the mean value of two determinations where the deviation from the mean is <10% of the mean value.
[b]Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 2. Anti-inflammatory activities for triarylpyrazoles **13a–p** and celecoxib.

	Mean change in edema thickness (% inhibition)		
Compound no.	1 h	3 h	5 h
Celecoxib	92.77*	97.77*	99.51*
13a	78.05*	86.71*	91.75*
13b	40.87*	70.42*	91.09*
13c	40.08*	82.73*	97.47*
13d	93.43*	97.18*	99.34*
13e	51.51*	83.32*	95.67*
13f	66.1*	95.92*	98.61*
13g	60.58*	88.46*	97.06*
13h	47.83*	85.16*	97.87*
13i	76.35*	93.21*	98.53*
13j	37.58*	92.14*	98.20*
13k	69.14*	91.37*	99.10*
13l	40.73*	80.11*	96.00*
13m	53.74*	74.20*	91.34*
13n	41.26*	86.52*	97.87*
13o	74.24*	97.18*	99.10*
13p	46.91*	86.61*	98.45*

*$p < 0.01$ significantly different from control at $p < 0.01$.

broad range (moderately potent to weekly potent) of COX-1 ($IC_{50} = 3.4–13.4$ μM), and COX-2 ($IC_{50} = 0.74–5.4$ μM range, see data in Table 1), inhibitory activities. All compounds were more potent inhibitors for COX-2 isozyme than COX-1 isozyme. Compounds having 4-$CH_3C_6H_4$ (**13e**, **13m**), 4-$OCH_3C_6H_4$ (**13f**, **13n**) and 2-furyl (**13h**, **13p**) moieties were generally more potent inhibitors of COX-2 and in turn more COX-2 selective (COX-2 S.I.=3.61–5.23) than compounds having the other moieties. The 4-methoxy derivative (**13f**) was the most potent COX-2 inhibitor ($IC_{50} = 0.74$ μM) while the 4-tolyl derivative (**13m**) was the most COX-2 selective (S.I.=5.23) in comparison with the reference drug celecoxib (COX-2 $IC_{50} = 1.3$ μM, S.I.=6.07).

In vivo *anti-inflammatory activity*

The anti-inflammatory activity of the all prepared triarylpyrazole derivatives (**13a–p**) were evaluated using carrageenan-induced rat paw edema assay. Each compound was administered orally (100 mg/kg) immediately prior to induction of inflammation by carrageenan subcutaneous injection. The anti-inflammatory activity was then calculated based on paw-volume changes at 1, 3 and 5 h after carrageenan injection as presented in Table 2.

A comparable study of the anti-inflammatory activity of the test compounds relative to celecoxib as a reference drug at different time intervals indicated that after 1 h, the triarylpyrazole derivatives (**13a–p**) showed edema inhibition percentage activities 37.58–93.43% and the 4-dimethylamino derivative (**13d**) was the most potent one (93.43% edema inhibition) while after 3 h, **13a–p** showed higher edema inhibition percentage activities 70.42–97.18% and both 4-dimethylamino derivative **13d** and 2-chloro derivative **13o** were the most potent derivatives (97.18% edema inhibition for both) (Table 3). After 5 h, all compounds showed higher edema inhibition percentage activities of 91.09–99.10 and the 4-dimethylamino derivative (**13d**) was still the highest potent derivative (99.10% edema inhibition). At all time intervals (1, 3 and 5 h), 4-dimethylamino derivative **13d**, 4-methoxy derivative **13f**, 4-fluoro derivative **13k** and 2-chloro derivative **13o** displayed good anti-inflammatory activities (93.43–99.34, 66.1–98.61, 69.14–99.10 and 74.24–99.10%, respectively) comparable to that of celecoxib (92.77–99.51%) and were the most potent derivatives among the 16 triarylpyrazole compounds (**13a–p**).

Table 3. The anti-inflammatory activity (ED_{50}, μmol/kg) of most potent triarylpyrazoles **13d**, **13f**, **13k**, **13o** and celecoxib.

Compound no.	ED_{50} (μmol/kg)[a]
13d	66.5
13f	73.4
13k	79.8
13o	70.5
Celecoxib	68.1

[a]Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the % inhibition of inflammation at 3 h after oral administration of the test compound at the specified dose (μmol/kg).

Moreover, the ED_{50} values for the most potent derivatives (**13d**, **13f**, **13k**, **13o**) were calculated in comparison with celecoxib (Figures 3–6). The four derivatives showed good anti-inflammatory activities ($ED_{50} = 66.5–79.8$ μmol/kg), while 4-dimethylamino derivative **13d** ($ED_{50} = 66.5$ μmol/kg) was more potent than celecoxib ($ED_{50} = 68.1$ μmol/kg), 4-methoxy derivative **13f**, 4-fluoro derivative **13k** and 2-chloro derivative **13o** were less potent but with acceptable ED_{50} values ($ED_{50} = 73.4$, 79.8 and 70.5 μmol/kg, respectively).

Ulcerogenic liability

The most potent anti-inflammatory compounds (**13d**, **13f**, **13k** and **13o**), low ulcerogenic reference drug (celecoxib) and high ulcerogenic reference drug (aspirin) were subjected to ulcerogenic liability at 100 mg/kg dose and the results are recorded in Table 4. It was clear that the tested compounds caused ulceration effect comparable to that of celecoxib (the relative ulcerogenicity of the tested compounds to celecoxib = 1.16–1.48). While the 4-dimethylamino derivative **13d** (ulcer index = 3.89) was the least ulcerogenic derivative in comparison with celecoxib (ulcer index = 3.35), the other three derivatives (**13f**, **13k**, **13o**) showed ulcerogenic potential (ulcer index = 4.86, 4.96 and 3.92, respectively) slightly higher than celecoxib (ulcer index = 3.35).

Figure 3. 2D binding interaction of **13d** inside COX-2 active site.

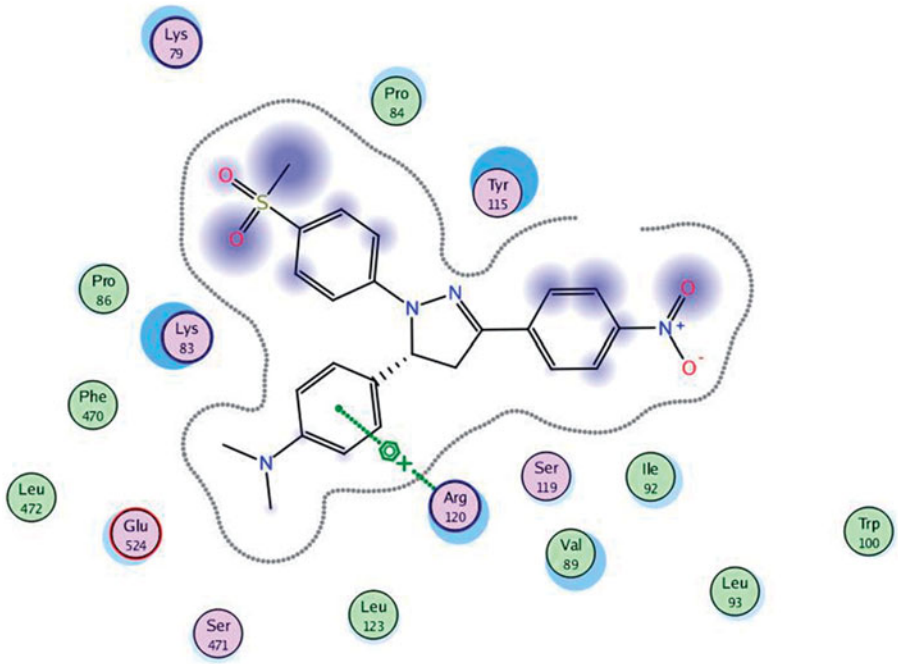
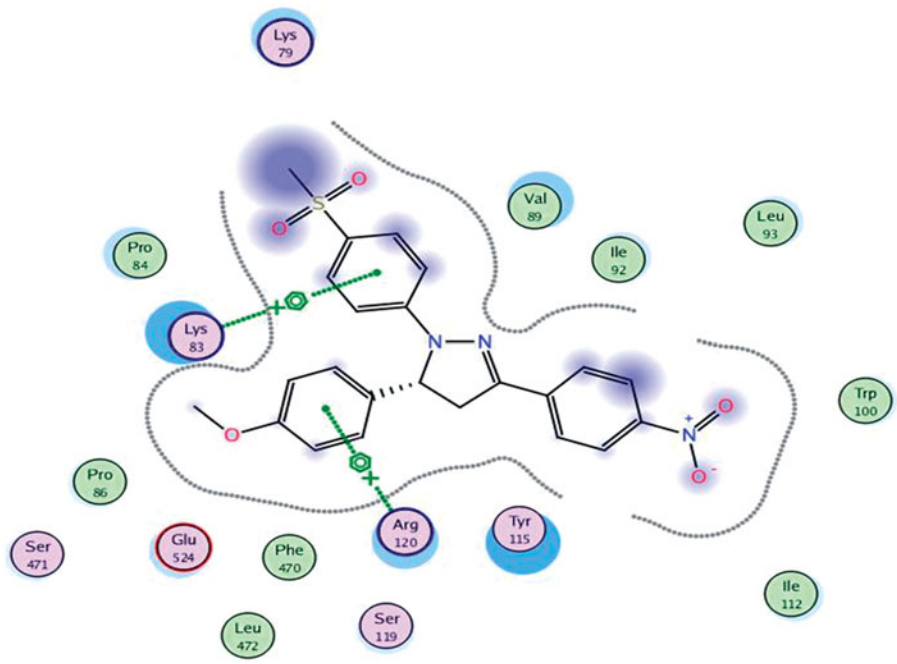


Figure 4. 2D binding interaction of **13f** inside COX-2 active site.



In comparison with the traditional NSAID aspirin (ulcer index = 22.75), all the tested compounds were about 4–6 folds less ulcerogenic with relative ulcerogenicity range of 0.17–0.22.

Molecular modeling

To know the plausible mode of interactions of the most potent anti-inflammatory compounds (**13d**, **13f**, **13k** and **13o**), molecular docking experiments were performed using X-ray crystal structure data for COX-2 obtained from the protein data bank using the COX-2 selective reference drug (celecoxib, **1**) as a ligand (Figure 7). The docking results including the energy associated with intermolecular interactions (affinity in kcal/mol) obtained upon computational docking for all compounds (**13d**, **13f**, **13k**, **13o** and celecoxib) within COX-2 active sites and the binding interactions between the amino acid residues and functional groups of the compounds are summarized in Table 5. While compounds **13d** and **13f** showed appreciable binding interactions (affinity in kcal/mol −6.9498 and −6.7965, respectively), compounds **13k** and **13o** showed binding interactions (affinity in kcal/mol −2.5760 and −2.1774, respectively) in comparison with celecoxib (affinity in kcal/mol −6.5330).

Conclusion

A new series of 1,3,5-triaryl-4,5-dihydro-1*H*-pyrazole derivatives **13a**–**p** were synthesized for evaluation as COX inhibitors, anti-inflammatory agents and ulcerogenic liability. Structure–activity data acquired and biological studies showed that (i) all compounds were more potent COX-2 inhibitors than COX-1, (ii) most

Figure 5. 2D binding interaction of **13k** inside COX-2 active site.

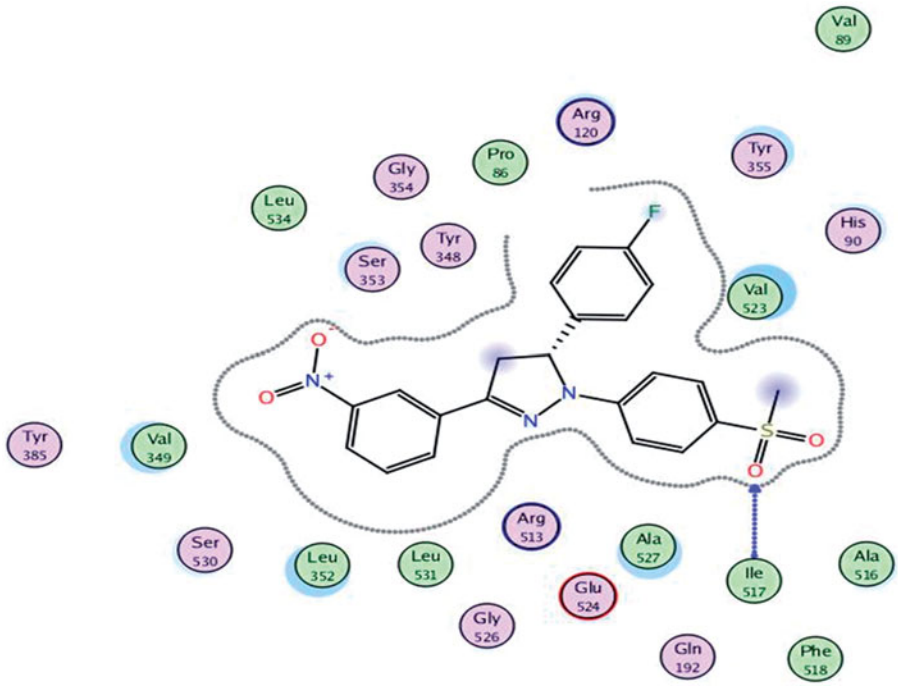


Figure 6. 2D binding interaction of **13o** inside COX-2 active site.

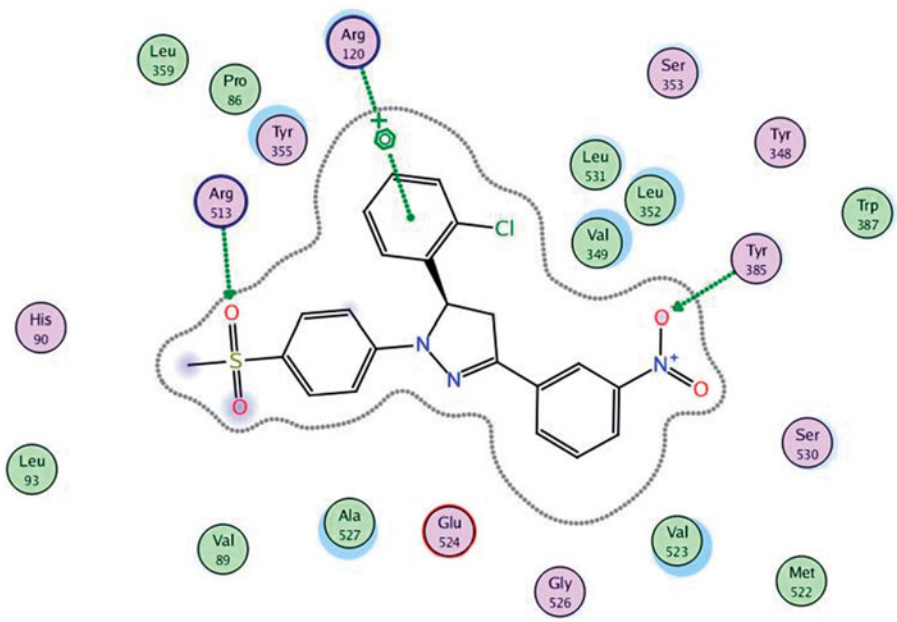


Table 4. Ulcerogenic effect of triarylopyrazoles **13d**, **13f**, **13k**, **13o**, aspirin and celecoxib.

Compound	Average severity	Average no of ulcers	% incidence/10	Ulcer index	Relative ulcerogenicity to celecoxib aspirin	
13d	0.63 ± 0.005	0.26 ± 0.003	3	3.89	1.16	0.17
13f	0.5 ± 0.025	0.36 ± 0.004	4	4.86	1.45	0.21
13k	0.61 ± 0.013	0.35 ± 0.006	4	4.96	1.48	0.22
13o	0.65 ± 0.004	0.27 ± 0.014	3	3.92	1.17	0.17
Aspirin	3.75	9	10	22.75	6.79	1
Celecoxib	0.6 ± 0.021	0.75 ± 0.022	2	3.35	1	0.15

Values represent means ± SEM of five animals for each group. Statistical analysis using one-way ANOVA.

Figure 7. 2D binding interaction of celecoxib inside COX-2 active site.

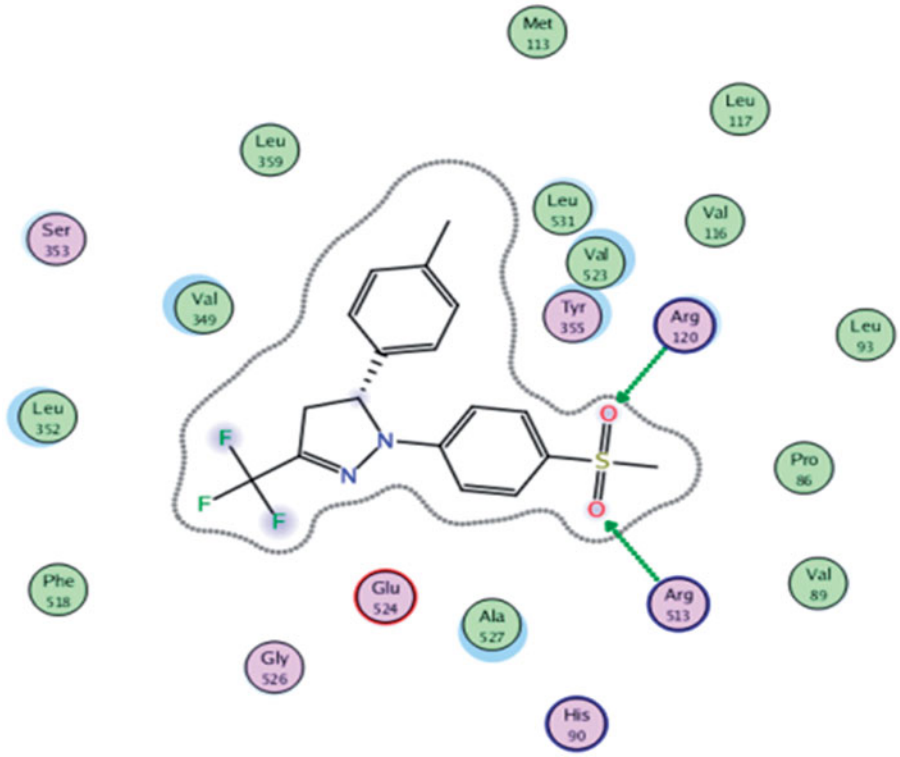


Table 5. Molecular modeling data for compounds **13d**, **13f**, **13k**, **13o** and celecoxib during docking in COX-2 (PDB ID: 1CX2) active site.

Compound	Affinity (kcal/mol)	Distance (in Å) from main residue		Functional group	Interaction
13d	−6.9498	4.27	Arg120	–Ph-ring	pi-cation
13f	−6.7965	4.02	Lys83	–Ph-ring	pi-cation
	4.37	Arg120	–Ph-ring	pi-cation	
13k	−2.5760	3.26	Ile517	$–SO_2$	H-acceptor
13o	−2.1774	2.75	Arg513	$–SO_2$	H-acceptor
	2.72	Tyr385	$–NO_2$	H-acceptor	
	4.28	Arg120	–Ph-ring	pi-cation	
Celecoxib	−6.5330	2.91	Arg513	$–SO_2$	H-acceptor
	2.51	Arg120	$–SO_2$	H-acceptor	

of the evaluated compounds showed good anti-inflammatory activity at different time intervals especially compounds **13d**, **13f**, **13k** and **13o**, (iii) while compound **13d** was more potent than celecoxib compounds, **13f**, **13k** and **13o** were less potent but with acceptable ED_{50} values and (iv) the four compounds (**13d**, **13f**, **13k** and **13o**) were 4–6 folds less ulcerogenic than aspirin and showed ulceration effect comparable to that of celecoxib.

Declaration of interest

The authors have declared no conflict of interest.

References

1. Nonato FR, Nogueira TMO, Barros TAA, et al. Antinociceptive and anti-inflammatory activities of *Adiantum latifolium* lam.: evidence for a role of IL-1β inhibition. J Ethnopharm 2011;136:518–24.
2. Al-Hourani BJ, Sharma SK, Mane JY, et al. Synthesis and evaluation of 1,5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors. Bioorg Med Chem Lett 2011;21:1823–6.
3. Ghodsi R, Zarghi A, Daraei B, Hedayati M. Design, synthesis and biological evaluation of new 2,3-diarylquinoline derivatives as selective cyclooxygenase-2 inhibitors. Bioorg Med Chem Lett 2010; 18:1029–33.
4. Rathish IG, Javed K, Ahmad S, et al. Synthesis and antiinflammatory activity of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide. Bioorg Med Chem Lett 2009;19:255–8.
5. Dogné JM, Supuran CT, Pratico D. Adverse cardiovascular effects of the coxibs. J Med Chem 2005;48:2251–7.
6. Abdellatif KRA, Chowdhury MA, Knaus EE. Synthesis of new 1-[4-methane (amino) sulfonylphenyl)]-5-[4-(aminophenyl)]-3-trifluoromethyl-1H-pyrazoles. J Het Chem 2008;45:1707–10.
7. Abdellatif KRA, Chowdhury MA, Dong Y, Knaus EE. Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 1-(4-methanesulfonylphenyl)-5-aryl-1H-pyrazol-3-carboxylic acids: synthesis, nitric oxide release studies and anti-inflammatory activities. Bioorg Med Chem 2008;16:6528–34.
8. Abdellatif KRA, Chowdhury MA, Dong Y, et al. Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1H-pyrazole and its methanesulfonyl analog: synthesis, biological evaluation and nitric oxide release studies. Bioorg Med Chem 2008;16:9694–8.
9. Chowdhury MA, Abdellatif KRA, Dong Y, Knaus EE. Synthesis of new 4-[2-(4-methyl(amino)sulfonylphenyl)-5-trifluoromethyl-2H-pyrazol-3-yl]-1,2,3,6-tetrahydropyridines: a search for novel nitric oxide donor anti-inflammatory agents. Bioorg Med Chem 2008;16: 8882–8.
10. Abdellatif KRA, Elshemy HAH, Salama. SA, Omar HA. Synthesis, characterization and biological evaluation of novel

4′-fluoro-2′-hydroxy-chalcones derivatives as antioxidant, anti-inflammatory and analgesic agents. J Enz Inhib Med Chem 2015;30:484–91.
11. Abdellatif KRA, Abdelwahab MA, Labib M, Zidan TH. Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of novel triarylpyrazoline derivatives as selective COX-2 inhibitors. Bioorg Med Chem Lett 2015;25:5787–91.
12. Ahlström MM, Redderström M, Zamora I, Luthman K. CYP2C9 structure – metabolism relationships: optimizing the metabolic stability of COX-2 inhibitors. J Med Chem 2007;50: 4444–52.
13. Romagnoli R, Baraldi PG, Carrion MD, et al. Hybrid alpha-bromoacryloylamido chalcones. Design, synthesis and biological evaluation. Bioorg Med Chem Lett 2009;19:2022–8.
14. Gupta RA, Kaskhedikar SG. Synthesis, antitubercular activity, and QSAR analysis of substituted nitroaryl analogs: chalcone, pyrazole, isoxazole, and pyrimidines. Med Chem Res 2013;22:3863–80.
15. Fun HK, Chantrapromma S, Patil PS, et al. (E)-3-(4-methyl-phenyl)-1-(4-nitro-phenyl)prop-2-en-1-one. Acta Crystallograph E Struct Reprts Online 2008;64:o954–5.
16. Indorkar D, Chourasia OP, Limaye SN. Synthesis and characterization of some new synthesis of 1-acetyl-3-(4-nitrophenyl)-5-(substituted phenyl) pyrazoline derivative and antimicrobial activity. Int J Curr Microbial APP Sci 2015;4:670–8.
17. Zheng CJ, Jiang SM, Chen ZH, et al. Synthesis and antibacterial activity of some heterocyclic chalcone derivatives bearing thiofuran, furan, and quinoline moieties. Archiv Pharm 2011;344: 689–95.
18. Mishra SK, Sahoo SP, Panda K, et al. Synthesis and antimicrobial activity of some 1-(2,4-dinitro-phenyl)-3-(3-nitrophenyl)-5-(4-substituted phenyl)-4-bromo-2-pyrazolines and 1-(2,4-dinitrophenyl)-3-(3-nitrophenyl)-5-(4-substituted phenyl)-2-pyrazoline-4-ones. Acta Pol Pharmaceut Drug Res 2007;64:359–64.
19. Qiu X, Li S, Shi A. Synthesis and biological activities of chalcones derived from nitroacetophenone. Adv Mater Res 2012;518:255–60.
20. Mageed IY, Jassim WK, Ahmed A, Jaasim IK. Synthesis and characterization of some new compounds including heterocyclic units. Aust J Basic Appl Sci 2014;8:404–11.
21. Balaji PN, Sai Sreevani M, Harini P, et al. Antimicrobial activity of some novel synthesized heterocyclic compounds from substituted chalcones. J Chem Pharm Res 2010;2:754–8.
22. Azam MA, Suresh B. Synthesis and biological evaluation of some novel 2-mercaptobenzothiazoles carrying 2-pyrazoline. J Sci Ind Res 2012;71:113–19.
23. Mamaghani M, Mahmoodi NO, Moghisseh AA, Pourmohamad L. Synthesis and kinetic resolution of furyl substituted secondary carbinols by porcine pancreatic lipase under solvent free conditions. J Iran Chem Soc 2008;5:238–43.
24. Abdellatif KRA, Chowdhury MA, Velázquez C, et al. Celecoxib prodrugs possessing a diazen-1-ium-1,2-diolate nitric oxide donor moiety: synthesis, biological evaluation and nitric oxide release studies. Bioorg Med Chem Lett 2010;20:4544–9.
25. Roschek BJ, Fink RC, Li D, et al. Pro-inflammatory enzymes, cyclooxygenase 1, cyclooxygenase 2, and 5-lipooxygenase, inhibited by stabilized rice bran extracts. J Med Food 2009;12:615–23.
26. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol 1962;111:544.
27. Hassan GS, Abou-Seri SM, Kamel G, Ali MM. Celecoxib analogs bearing benzofuran moiety as cyclooxygenase-2 inhibitors: design, synthesis and evaluation as potential anti-inflammatory agents. Eur J Med Chem 2014;76:482–93.
28. Wang JL, Limburg D, Graneto MJ, et al. The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: The second clinical candidate having a shorter and favorable human half-life. Bioorg Med Chem Lett 2010;20:7159–63.

Supplementary material available online