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RESEARCH ARTICLE

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

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Abstract A novel series of 2-pyrazoline derivatives **13a–l** was synthesized via aldol condensation of 4-substituted acetophenones with appropriately substituted aldehydes followed by cyclization of the formed chalcones with 4-hydrazinobenzenesulfonamide hydrochloride. The chemical structures of the target pyrazoline derivatives were proved by means of IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analyses data. All the synthesized compounds were evaluated for their cyclooxygenase selectivity, anti-inflammatory and ulcerogenic liability. While compounds **13e**, **13h** and **13i** showed moderate COX-2 selectivity in vitro and good anti-inflammatory activity in vivo, compound **13i** showed the highest anti-inflammatory activity that is very close in potency to the reference drug (celecoxib) with better gastric profile than celecoxib.

Keywords Dihydropyrazole · Cyclooxygenase inhibition · Anti-inflammatory

Introduction

The anti-inflammatory activity of non-steroidal anti-inflammatory drugs (NSAIDs) results from the inhibition of cyclooxygenase enzyme which catalyze the production of pro-inflammatory prostaglandins (PGs) and thromboxanes (TXs) (Zarghi et al. 2007; Zebardast et al. 2009). It is well known that cyclooxygenase enzyme exists in two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2). The constitutive COX-1 plays a critical role as a housekeeping enzyme which is responsible for the maintenance of physiological functions such as protection of gastric mucosa, vascular homeostasis and platelet aggregation. The inducible COX-2 is significantly upregulated during acute and chronic inflammation, pain and oncogenesis (Rathish et al. 2009; Al-Hourani et al. 2011). Traditional NSAIDs such as aspirin, ibuprofen and indomethacin interact with both forms (COX-1 and COX-2) leading to the inhibition of gastro protective PGs synthesized through COX-1 pathway and therefore their long term administration even at low prophylactic doses resulting in gastrointestinal side effects ranging from ulcers to perforation and bleeding (Zarghi et al. 2009). For this reason, the synthesis of selective COX-2 inhibitor drugs (coxibs) takes much consideration in recent years that achieve the same anti-inflammatory efficacy as traditional NSAIDs, but with minimal risk of unwanted gastrointestinal side effects mediated through the inhibition of COX-1 enzyme (Rathish et al. 2009). Celecoxib (**1**), rofecoxib (**2**) and valdecoxib (**3**) are the most common coxibs approved for marketing (Fig. 1). However, adverse biochemical changes in the COX pathway caused by highly selective COX-2 inhibitors are believed to be responsible for the increased incidences of high blood pressure and myocardial infarction that led to the withdrawal of rofecoxib and valdecoxib from market (Scheen 2004; Dogné et al. 2005). In this context, celecoxib is one of the most important highly marketed COX-2 selective drugs till now (Ovais et al. 2012). In earlier studies, we reported some derivatives of celecoxib (**4**–**8**) (Abdellatif et al. 2008a, b, c; Chowdhury et al. 2008a, b; Abdellatif et al. 2009) (Fig. 1) having comparable activities with celecoxib as COX-2 selective compounds with maintaining the pyrazole nucleus of celecoxib. But due to serious side effects such as water and salt

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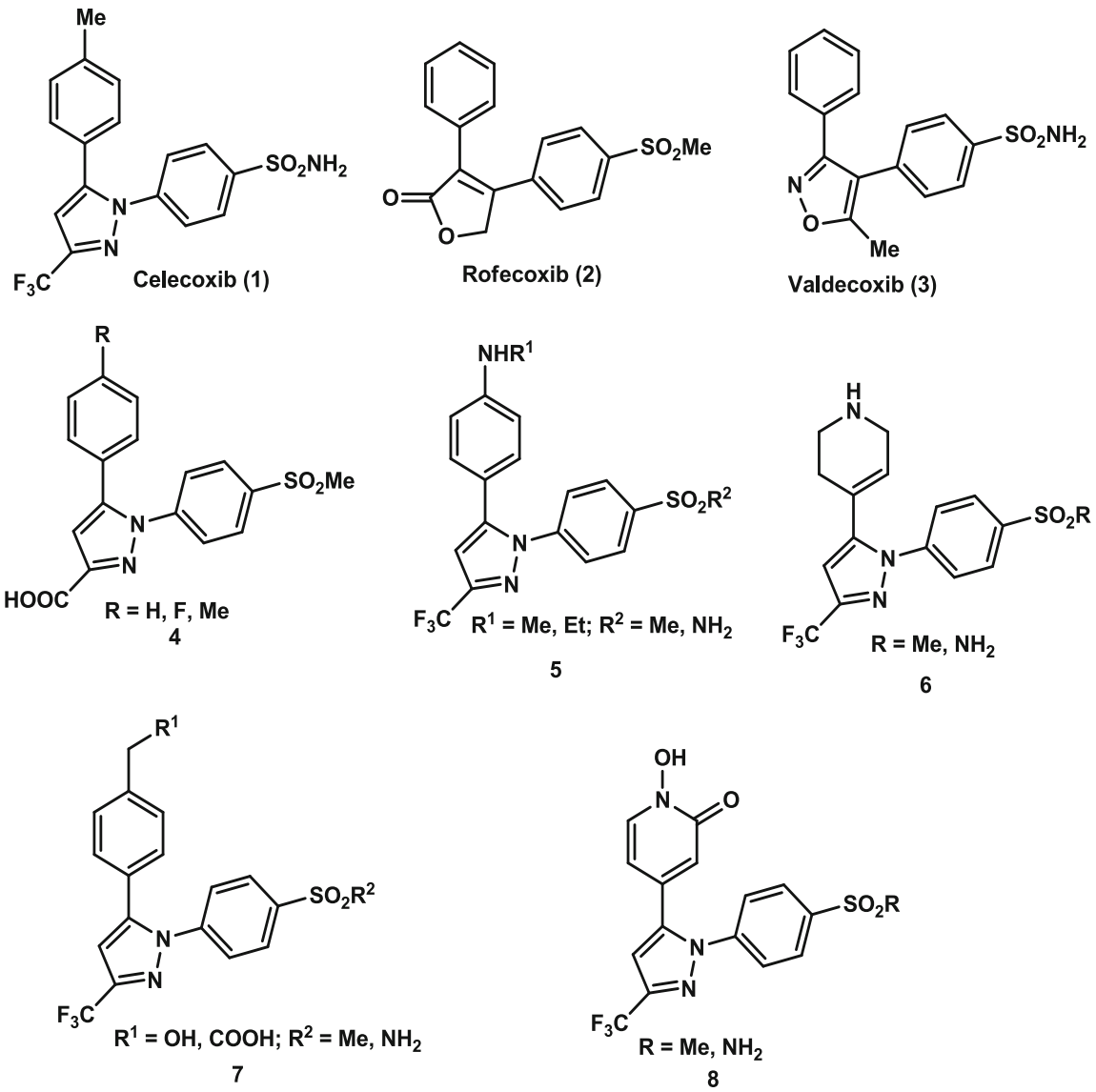

Fig. 1 Chemical structures of some selective cyclooxygenase-2 (COX-2) inhibitors (1–8)

retention, bone marrow depression and carcinogenesis, the use of pyrazole derivatives has been limited (Fioravanti et al. 2010). This limitation has led to the investigation of novel pyrazole derivatives with more potent anti-inflammatory activity and less gastrointestinal toxicity. Many selective COX-2 inhibitors belong to a group of compounds having a central tri-substituted planar ring, five-membered heterocyclic ring, attached to pendent benzene rings and a lipophilic group. One of the phenyl rings is bearing SO_2NH_2 moiety which is believed to induce COX-2 selectivity (Habeeb et al. 2001). 2-Pyrazoline ring system is an important nitrogenous five-membered heterocyclic component of the drugs and has attracted significant interest in medicinal chemistry over the past few decades. Literature survey declared that many pyrazoline derivatives have found their clinical application as NSAIDs. For example, Antipyrine, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, was found to be the first pyrazoline derivative used in the management of pain and inflammation. In addition, several related analogues of pyrazolidin-3,5-diones, pyrazolin-3-ones and pyrazolin-5-ones are also available as NSAIDs; examples are felcobuzone, mefobutazone, morazone, famprofazone, and ramifenazone (Khode et al. 2009). Besides these, many scaffolds containing the 2-pyrazoline,4,5-dihydropyrazole, heterocyclic are also reported in literature as having potent anti-inflammatory (Rathish et al. 2009; Bano et al. 2011; Bashir et al. 2011; Ovais et al. 2012, 2013; Khode et al. 2009), antidepressant, anti-bacterial (Johnson et al. 2007), anticancer (Rostom 2006; Johnson et al. 2007), anti-tubercular, analgesic (Sahu et al. 2008; Chandra et al. 2010), antidiabetic (Ahn et al. 2004) and CB1 receptor antagonism for obesity (Lange et al. 2005). So, pyrazolines are considered

useful lead molecules as they show significant pharmacological properties and clinical applications. Due to the great potential of both moieties, benzenesulfonamide and pyrazoline, the synthesis of pyrazolines bearing benzenesulfonamide was carried out to evaluate their anti-inflammatory activities.

Motivated by aforesaid findings and as a part of our ongoing research, we now describe the synthesis and anti-inflammatory (AI) activity for a new group of 1,3,5-triaryl-4,5-dihydro-1*H*-pyrazoles **13a–l** as celecoxib derivatives in which; (i) pyrazole ring of celecoxib was replaced with dihydropyrazole nucleus, (ii) trifluoromethyl moiety was replaced with 4-substituted aryl moiety since it was reported that the substituent at pyrazole C-3 has very few steric restrictions with respect to COX-2 suggesting that COX-2 inhibition should be retained (Ahlström et al. 2007), (iii) tolyl group was maintained or replaced with different aryl or heteroaryl moieties and (iv) aminosulfonyl group on the N^1-phenyl ring was maintained because aminosulfonyl or methylsulfonyl group is essential for potent and selective COX-2 inhibitory activity.

Materials and methods

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. 1H 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 and 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, were used without further purification; **11a** (Romagnoli et al. 2009), **11b** (Fun et al. 2008), **11c**,**d** (Gupta and Kaskhedikar 2012), **11e**,**f** (Zheng et al. 2011), **11g–j** (Amutha and Nagarajan 2012) and **12** (Abdellatif et al. 2010) were prepared according to reported procedures.

*General method for preparation of 1,3-diarylprop-2-en-1-ones (**11k**,**l**)*

4-isobutylacetophenone (**9b**, 2.0 mmol, 0.352 g) and the appropriate hetero aromatic aldehyde **10e** or **10f** (2.0 mmol) were dissolved in methanol (10 mL). An aqueous solution of sodium hydroxide (5 % w/v, 5 mL) was added slowly and the reaction mixture was stirred at room temperature for 12 h. The obtained solid was filtered, washed with water (5 mL) and crystallized from methanol to yield analytically pure compounds **11k** and **11l** for which physical and spectral data are listed below.

*3-(Fur-2-yl)-1-(4-isobutylphenyl)prop-2-en-1-one (**11k**)* Yield: 75 %; mp 54–56 °C; IR (KBr): 3037 (CH aromatic), 2923 (CH aliphatic), 1656 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (d, J = 6.4 Hz, 6H, $CH_2CH(CH_3)_2$), 1.83 (m, 1H, $CH_2CH(CH_3)_2$), 2.47 (d, J = 7.2 Hz, 2H, $CH_2CH(CH_3)_2$), 6.66 (dd, J = 3.4, 1.8 Hz, 1H, furyl H-4), 7.08 (d, J = 3.4 Hz, 1H, furyl H-5), 7.28 (d, J = 7.6 Hz, 2H, isobutylphenyl H-3, H-5), 7.55 (d, J = 15.2 Hz, 1H, COCH = CH), 7.85 (d, J = 15.2 Hz, 1H, COCH = CH), 7.89 (d, J = 1.8 Hz, 1H, furyl H-3), 7.97 (d, J = 7.6 Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.5, 29.8, 44.8, 113.5, 117.2, 119.1, 128.6, 129.6, 130.4, 135.4, 146.4, 147.3, 151.6, 188.4; MS (m/z): 254 ($M^{+\cdot}$, 75 %), 211 (100 %); Anal. Calcd for $C_{17}H_{18}O_2$: C, 80.28; H, 7.13. Found: C, 80.45; H, 6.87.

*1-(4-Isobutylphenyl)-3-(thien-2-yl)-prop-2-en-1-one (**11l**)* Yield: 70 %; mp 60–62 °C; IR (KBr): 3072 (CH aromatic), 2920 (CH aliphatic), 1648 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.85 (d, J = 6.4 Hz, 6H, $CH_2CH(CH_3)_2$), 1.86 (m, 1H, $CH_2CH(CH_3)_2$), 2.52 (d, J = 7.2 Hz, 2H, $CH_2CH(CH_3)_2$), 7.19 (dd, J = 4.8, 3.2 Hz, 1H, thienyl H-4), 7.32 (d, J = 7.6 Hz, 2H, isobutylphenyl H-3, H-5), 7.56 (d, J = 15.2 Hz, 1H, COCH = CH), 7.67 (d, J = 3.2 Hz, 1H, thienyl H-3), 7.77 (d, J = 4.8 Hz, 1H, thienyl H-5), 7.91 (d, J = 15.2 Hz, 1H, COCH = CH), 8.01 (d, J = 7.6 Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.5, 30.0, 44.9, 120.8, 128.7, 129.1, 129.8, 130.7, 133.1, 135.7, 136.8, 140.2, 147.4, 188.5; MS (m/z): 270 ($M^{+\cdot}$, 100 %), 192 (100 %); Anal. Calcd for $C_{17}H_{18}OS$: C, 75.51; H, 6.71. Found: C, 75.68; H, 6.49.

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

To a solution of the appropriate chalcone **11a–l** (2.0 mmol) in ethanol (20 mL), 4-hydrazinobenzenesulfonamide hydrochloride (**12**, 2.4 mmol, 0.535 g) was added and the reaction mixture was refluxed 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 pyrazoles **13a–l**. Physical and spectral data for **13a–l** are listed below.

1-(4-Aminosulfonylphenyl)-3-(4-nitrophenyl)-5-(phenyl)-4,5-dihydro-1H-pyrazole (***13a***) Yield: 85 %; mp 200–202 °C; IR (KBr): 3397 (NH_2), 2922 (CH aliphatic), 1590 (C=N), 1354, 1149 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.27 (dd, $J = 17.6$, 4.8 Hz, 1H, pyrazole H-4), 4.03 (dd, $J = 17.6$, 12 Hz, 1H, pyrazole H′-4), 5.77 (dd, $J = 12$, 4.8 Hz, 1H, pyrazole H-5), 7.08 (s, 2H, NH_2, D_2O exchangeable), 7.16 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.27 (d, $J = 6.8$ Hz, 2H, phenyl H-2, H-6), 7.36 (t, J = 6.8 Hz, 3H, phenyl H-3, H-4, H-5), 7.62 (d, $J = 8.4$ Hz, aminosulfonylphenyl H-3, H-5), 8.01 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-2, H-6), 8.28 (d, $J = 8.4$ Hz,2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 42.8, 63.4, 113.1, 124.4, 126.1, 127.2, 127.6, 128.4, 129.7, 133.9, 138.4, 141.4, 145.7, 147.4, 148.1; EIMS: m/z (%): 422 ($M^{+\cdot}$, 40), 80 (100); Anal. Calcd for $C_21H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26. Found: C, 59.33; H, 3.95; N, 13.00.

1-(4-Aminosulfonylphenyl)-5-(4-methylphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (***13b***) Yield: 90 %; mp 223–225 °C; IR (KBr): 3433, 3332 (NH_2), 2919 (CH aliphatic), 1587 (C=N), 1327 and 1154 (SO_2); ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3H, CH_3) 3.22 (dd, $J = 17.6$, 4.8 Hz, 1H, pyrazole H-4), 3.99 (dd, $J = 17.6$, 12.4 Hz, 1H, pyrazole H′-4), 5.71 (dd, $J = 12.4$, 4.8 Hz, 1H, pyrazole H-5), 7.09 (s, 2H, NH_2, D_2O exchangeable), 7.15 (m, 4H, methylphenyl H-2, H-3, H-5, H-6), 7.17 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.63 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.99 (d, $J = 8.4$ Hz, nitrophenyl H-2, H-6), 8.26 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 20.8, 42.6, 63.1, 113.1, 124.3, 125.9, 126.9, 127.5, 130.1, 133.3, 137.9, 138.2, 145.8, 147.2, 148.0; EIMS: m/z (%): 436 (M^+, 100); Anal. Calcd for $C_{22}H_{20}N_4O_4S$: C, 60.54; H, 4.62; N, 12.84. Found: C, 60.19; H, 4.35; N, 12.59.

1-(4-Aminosulfonylphenyl)-5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (***13c***) Yield: 78 %; mp 233–235 °C; IR (KBr): 3426, 3347 (NH_2), 2919 (CH aliphatic), 1585 (C=N), 1331, 1155 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.28 (dd, $J = 17.6$, 4.8 Hz, 1H, pyrazole H-4), 4.03 (dd, $J = 17.6$, 12.4 Hz, 1H, pyrazole H′-4), 5.80 (dd, $J = 12.4$, 4.8 Hz, 1H, pyrazole H-5), 7.09 (s, 2H, NH_2, D_2O exchangeable), 7.16 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.29 (d, $J = 8.4$ Hz, 2H, chlorophenyl H-2, H-6), 7.42 (d, $J = 8.4$ Hz, 2H, chlorophenyl H-3, H-5), 7.63 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.01 (d, $J = 8.4$ Hz, nitrophenyl H-2, H-6), 8.28 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 42.6, 62.7, 113.1, 124.4, 127.3, 127.6, 128.2, 129.6, 132.8, 134.2, 138.3, 140.4, 145.5, 147.5, 148.1; EIMS: m/z (%): 456 ($M^{+\cdot}$, 100); Anal. Calcd for $C_21H_{17}ClN_4O_4S$: C, 55.20; H, 3.75; N, 12.26. Found: C, 55.01; H, 3.43; N, 12.37.

1-(4-Aminosulfonylphenyl)-5-(4-fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (***13d***) Yield: 77 %; mp 180–182 °C; IR (KBr): 3420 (NH_2, broad), 2916 (CH aliphatic), 1592 (C=N), 1333, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.27 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 4.02 (dd, $J = 17.6$, 12 Hz, 1H, pyrazole H′-4), 5.80 (dd, $J = 12$, 4.4 Hz, 1H, pyrazole H-5), 7.10 (s, 2H, NH_2, D_2O exchangeable), 7.17 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.32 (m, 4H, fluorophenyl H-2, H-3, H-5, H-6), 7.63 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.01 (d, $J = 8.4$ Hz, nitrophenyl H-2, H-6), 8.28 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 42.7, 62.7, 113.2, 116.2, 116.5, 124.3, 127.1, 127.6, 128.21, 128.29, 137.5, 138.2, 145.7, 147.4, 148.1; EIMS: m/z (%): 440 ($M^{+\cdot}$, 46), 83 (100); Anal. Calcd for $C_21H_{17}FN_4O_4S$: C, 57.27; H, 3.89; N, 12.72. Found: C, 57.51; H, 3.94; N, 12.97.

1-(4-Aminosulfonylphenyl)-5-(fur-2-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (***13e***) Yield: 75 %; mp 220–222 °C; IR (KBr): 3362, 3269 (NH_2), 1590 (C=N), 1340, 1153 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.27 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 4.02 (dd, $J = 17.6$, 12 Hz, 1H, pyrazole H′-4), 5.80 (dd, $J = 12$, 4.4 Hz, 1H, pyrazole H-5), 6.39 (dd, $J = 3.3$, 1.8 Hz, 1H, furyl H-4), 6.54 (d, $J = 3.3$ Hz, 1H, furyl H-5), 7.08 (s, 2H, NH_2, D_2O exchangeable), 7.3 (d, $J = 9$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.55 (d, $J = 0.9$ Hz, 1H, furyl H-3), 7.66 (d, $J = 9$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.03 (d, $J = 8.7$ Hz, nitrophenyl H-2, H-6), 8.29 (d, $J = 8.7$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 39.0, 57.0, 109.1, 110.9, 113.3, 124.4, 127.2, 127.5, 134.9, 138.4, 143.7, 145.6, 147.5, 148.3, 152.3; EIMS: m/z (%): 412 (M^+, 21), 106 (100); Anal. Calcd for $C_19H_{16}N_4O_5S$: C, 55.33; H, 3.91; N, 13.58. Found: C, 55.47; H, 3.98; N, 13.69.

1-(4-Aminosulfonylphenyl)-5-(thien-2-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (***13f***) Yield: 85 %; mp 208–210 °C; IR (KBr): 3396, 3282 (NH_2), 1590 (C=N), 1323, 1154 (SO_2); ^{1}H NMR (DMSO-d_6) δ 3.43 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 3.99 (dd, $J = 17.6$, 12.4 Hz, 1H, pyrazole H′-4), 6.16 (dd, $J = 12.4$, 4.4 Hz, 1H, pyrazole H-5), 6.96 (dd, $J = 4.4$, 3.2 Hz, 1H, thienyl H-4), 7.15 (s, 2H, NH_2, D_2O exchangeable), 7.19 (d, $J = 3.2$ Hz, 1H, thienyl H-3), 7.28 (d, $J = 8.4$ Hz, 2H, minosulfonylphenyl H-2, H-6), 7.40 (d, $J = 4.8$, 1H, thienyl H-5), 7.67 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.03 (d, $J = 8.4$ Hz, nitrophenyl H-2, H-6), 8.29 (d, $J = 8.4$ Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6) δ 42.9, 59.4, 113.5, 124.4, 126.2, 126.3, 127.2, 127.5, 134.3, 138.2, 144.1, 145.7, 147.5, 148.3; EIMS: m/z (%): 428 (M^+, 32), 106 (100); Anal.

Calcd for $C_{19}H_{16}N_4O_4S_2$: C, 53.26; H, 3.76; N, 13.08. Found: C, 53.43; H, 3.80; N, 13.21.

1-(4-Aminosulfonylphenyl)-3-(4-isobutylphenyl)-5-(phenyl)-4,5-dihydro-1H-pyrazole ***(13g)*** Yield: 80 %; mp 108–110 °C; IR (KBr): 3382, 3267 (NH_2), 2954, 2923 (CH aliphatic), 1591 (C=N), 1331 and 1156 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.87 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.46 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$), 3.17 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 3.96 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.62 (dd, $J = 11.6$, 4.4 Hz, 1H, pyrazole H-5), 7.02 (s, 2H, NH_2, D_2O exchangeable), 7.06 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.25 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.33 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-3, H-5), 7.58 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.70 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.4, 30.0, 43.0, 44.7, 62.1, 112.4, 124.7, 126.4, 127.5, 127.7, 129.2, 129.8, 133.1, 143.6, 146.2, 147.3, 149.4, 150.4; EIMS: m/z (%): 433 ($M^{+\cdot}$, 58), 64 (100); Anal. Calcd for $C_{25}H_{27}N_3O_2S$: C, 69.26; H, 6.28; N, 9.69. Found: C, 68.97; H, 6.39; N, 9.51.

1-(4-Aminosulfonylphenyl)-3-(4-isobutylphenyl)-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazole ***(13h)*** Yield: 75 %; mp 140–142 °C; IR (KBr): 3396, 3267 (NH_2), 2953, 2919 (CH aliphatic), 1592 (C=N), 1330, 1154 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.87 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.24 (s, 3H, CH_3), 2.50 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$),3.13 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 3.92 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.57 (dd, $J = 11.6$, 4.4 Hz, 1H, pyrazole H-5), 7.01 (s, 2H, NH_2, D_2O exchangeable), 7.05 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.13 (m, 4H, methylphenyl H-2, H-3, H-5, H-6), 7.23 (d, J = 7.6 Hz, 2H, isobutylphenyl H-3, H-5), 7.57 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.69 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 20.9, 22.4, 29.9, 43.4, 44.7, 62.4, 112.3, 125.9, 126.2, 127.5, 129.5, 129.8, 130.0, 132.3, 137.5, 138.8, 143.4, 146.5, 150.4; EIMS: m/z (%): 447 (M^+, 32), 64 (100); Anal. Calcd for $C_{26}H_{29}N_3O_2S$: C, 69.77; H, 6.53; N, 9.39. Found: C, 69.94; H, 6.69; N, 9.47.

1-(4-Aminosulfonylphenyl)-5-(4-chlorophenyl)-3-(4-isobutylphenyl)-4,5-dihydro-1H-pyrazole ***(13i)*** Yield: 85 %; mp 110–112 °C; IR (KBr): 3381, 3263 (NH_2), 2952 (CH aliphatic), 1591 (C=N), 1326, 1152 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.87 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.49 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$), 3.18 (dd, $J = 17.6$, 4.4 Hz, 1H, pyrazole H-4), 3.95 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.65 (dd, $J = 11.6$, 4.4 Hz, 1H, pyrazole H-5), 7.02 (s, 2H, NH_2, D_2O exchangeable), 7.06 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.24 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-3, H-5), 7.27 (d, $J = 8$ Hz, 2H, chlorophenyl H-2, H-6), 7.41 (d, $J = 8$ Hz, 2H, chlorophenyl H-3, H-5) 7.59 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.70 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.4, 29.9, 43.2, 44.7, 62.0, 112.4, 117.7, 126.3, 127.6, 128.0, 129.5, 129.8, 132.7, 140.8, 143.5, 146.3, 150.4; EIMS: m/z (%): 467 (M^+, 60), 468 (M^{+1}, 22), 469 (M^{+2}, 24), 64 (100 %); Anal. Calcd for $C_{25}H_{26}ClN_3O_2S$: C, 64.16; H, 5.60; N, 8.98. Found: C, 64.50; H, 5.39; N, 9.09.

1-(4-Aminosulfonylphenyl)-5-(4-fluorophenyl)-3-(4-isobutylphenyl)-4,5-dihydro-1H-pyrazole ***(13j)*** Yield: 76 %; mp 148–150 °C; IR (KBr): 3381, 3261 (NH_2), 3073 (CH aromatic), 2956, 2871 (CH aliphatic), 1595 (C=N), 1333, 1157 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.89 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.47 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$), 3.17 (dd, $J = 17.6$, 4.8 Hz, 1H, pyrazole H-4), 3.95 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.65 (dd, $J = 11.6$, 4.8 Hz, 1H, pyrazole H-5), 7.04 (s, 2H, NH_2, D_2O exchangeable), 7.06 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.17 (t, $J = 8.4$ Hz, 2H, fluorophenyl H-3, H-5),7.24 (d, $J = 8$ Hz, 2H, isobutylphenyl H-3, H-5), 7.29 (d, $J = 8.4$ Hz, 2H, fluorophenyl H-2, H-6), 7.59 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.70 (d, $J = 8$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.4, 29.9, 43.3, 44.7, 61.9, 112.3, 116.1, 116.4, 126.3, 127.6, 128.1, 129.8, 132.6, 143.5, 146.4, 150.4, 160.6, 163.0; EIMS: m/z (%): 451 (M^+, 32), 80 (100); Anal. Calcd for $C_{25}H_{26}FN_3O_2S$: C, 66.50; H, 5.80; N, 9.31. Found: C, 66.34; H, 5.80; N, 9.35.

1-(4-Aminosulfonylphenyl)-5-(fur-2-yl)-3-(4-isobutylphenyl)-4,5-dihydro-1H-pyrazole ***(13k)*** Yield: 95 %; mp 140–142 °C; IR (KBr): 3381, 3260 (NH_2), 2954, 2923(CH aliphatic), 1591 (C=N), 1329, 1155 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.89 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.47 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$), 3.17 (dd, $J = 17.6$, 4.8 Hz, 1H, pyrazole H-4), 3.95 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.65 (dd, $J = 11.6$, 4.8 Hz, 1H, pyrazole H-5), 6.38 (dd, $J = 3.4$, 1.8 Hz, 1H, furyl H-4), 6.48 (d, $J = 3.4$ Hz, 1H, furyl H-5), 7.07 (s, 2H, NH_2, D_2O exchangeable), 7.20 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.25 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-3, H-5), 7.55 (d, $J = 1.8$ Hz, 1H, furyl H-3), 7.63 (d, $J = 8.4$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.72 (d, $J = 7.6$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.5, 30.0, 39.3, 44.8, 56.2, 108.6, 110.8, 112.5, 126.4, 127.5,

128.7, 129.7, 133.6, 143.2, 143.4, 146.5, 150.4, 152.8; EIMS: m/z (%): 423 (M^+, 41), 106 (100); Anal. Calcd for $C_{23}H_{25}N_3O_3S$: C, 65.23; H, 5.95; N, 9.92. Found: C, 65.58; H, 5.69; N, 10.06.

1-(4-Aminosulfonylphenyl)-3-(4-isobutylphenyl)-5-(thien-2-yl)-4,5-dihydro-1H-pyrazole ***(13l)*** Yield: 80 %; mp 120–122 °C; IR (KBr): 3365, 3261 (NH_2), 3101 (CH aromatic), 2954, 2919 (CH aliphatic), 1590 (C=N), 1329, 1155 (SO_2); ^{1}H NMR (DMSO-d_6) δ 0.88 (d, $J = 6.4$ Hz, 6H, $CH_2CH(CH_3)_2$), 1.86 (m, 1H, $CH_2CH(CH_3)_2$), 2.46 (d, $J = 7.2$ Hz, 2H, $CH_2CH(CH_3)_2$), 3.32 (dd, $J = 18$, 4.4 Hz, 1H, pyrazole H-4), 3.92 (dd, $J = 17.6$, 11.6 Hz, 1H, pyrazole H′-4), 5.99 (dd, $J = 11.6$, 4.4 Hz, 1H, pyrazole H-5), 6.95 (dd, $J = 4.8$, 3.6 Hz, 1H, thienyl H-4), 7.06 (s, 2H, NH_2, D_2O exchangeable), 7.15 (d, $J = 3.6$ Hz, 1H, thienyl H-3), 7.19 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.25 (d, $J = 8.0$ Hz, 2H, isobutylphenyl H-3, H-5), 7.38 (d, $J = 4.8$ Hz, thienyl H-5), 7.62 (d, $J = 8.8$ Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.72 (d, $J = 8.0$ Hz, 2H, isobutylphenyl H-2, H-6); ^{13}C NMR (DMSO-d_6) δ 22.5, 30.0, 43.5, 44.8, 58.8, 112.8, 125.9, 126.0, 126.4, 127.4, 127.5, 129.5, 129.8, 133.3, 143.5, 144.8, 146.6, 150.7; EIMS: m/z (%): 439 ($M^{+\cdot}$, 54), 64 (100); Anal. Calcd for $C_{23}H_{25}N_3O_2S_2$: C, 62.84; H, 5.73; N, 9.56. Found: C, 62.61; H, 5.37; N, 9.25.

Biological evaluation

COX-1/COX-2 inhibition colorimetric assay

The ability of the tested compounds to inhibit COX-1/COX-2 was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Kit catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and as mentioned before (Abdelazeem et al. 2014). Different concentrations of tested compounds or celecoxib were incubated with the enzymes for a period of 5 min at 25 °C. After the incubation period and the addition of colorimetric substrate and arachidonic acid, the absorbance was measured at 590 nm using plate reader.

Anti-inflammatory activity

Animals

Adult male Wister albino rats (125–150 g) were used throughout the study of anti-inflammatory activity and ulcerogenic liabilities. The animals (five per cage) were kept at controlled conditions (temperature 23 ± 2 °C, humidity 60 ± 10 %) and a 12/12 h light/dark cycle with access to food and water. All procedures relating to animal care and treatments were performed according to protocols approved by the Research Ethical Committee of Faculty of Pharmacy Beni-Suef University (2014-Beni-Suef, Egypt).

Formalin induced rat paw edema

Anti-inflammatory activity study for the prepared compounds **13a–l** was determined in vivo by conducting the standard formalin-induced paw edema method in rats (Turner 1965). Wister albino rats were divided into forty groups of five animals each. Thickness of the left hind paw of each rat was measured in millimeters using plethysomometer, before any drug administration for each rat (0 h). The first group was kept as a negative control given 10 % aqueous solution of DMSO (v/v). The second, third and fourth groups were orally administered celecoxib as a reference standard in three different doses, one dose for each group, to calculate ED_{50}. The rest groups were treated orally with the tested compounds **13a–l**, three groups for each compound, in the form of 10 % aqueous solution of DMSO. Each compound was administered in three different concentrations ranging from 20 to 200 mg/kg body weight to calculate ED_{50} for each compound and treatment began 1 h before induction of inflammation. Induction of paw edema was performed by subcutaneous injection of 2.5 % of formalin (0.1 mL/rat) into the right hind paw of each rat. Paw thickness of each rat was measured after 3 h of formalin injection using plethysomometer.

Ulcerogenic liability

The ulcerogenic effect of **13a–l** as well as celecoxib was evaluated according to Meshali's method (Hassan et al. 2014). Seventy adult male albino rats were used in this study and divided into 14 groups. The animals were allowed to fast for 18 h before the administration of the drug. The first group received 10 % aqueous solution of DMSO (v/v) and served as a control group. The second group received celecoxib as a reference drug in a dose of 50 mg/kg. The other groups received the prepared compounds **13a–l** in a dose of 50 mg/kg. After 2 h of administration of drug animals were fed. Rats were given the required dose orally for three successive days. After 2 h of the last given dose, rats were sacrificed; the stomach of each rat was removed then opened along the greater curvature and rinsed with sodium chloride 0.9 %. In order to examine the stomach, it was stretched by pins on a corkboard. With the aid of a magnifying lens (10 xs) ulcers and erosions were searched in the stretched stomach. Calculation of the ulcer index was done according to Robert's method (Hassan et al. 2014). The expression of the degree of ulcerogenic effect was in term of dividing the percentage incidence of ulcers in each group of animal by ten, 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 one way ANOVA followed by Dunnett'stest. The results were expressed as mean ± standard deviation (SD). Differences were considered significant at $*P < 0.05$, and $**P < 0.01$, respectively.

Results and discussion

Chemistry

A group of 3-(4-isobutyl(nitro)phenyl)-5-(substituted aryl)-1-(4-sulphamoylphenyl) 4,5-dihydro-1*H*-pyrazoles (**13a–l**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, the reaction of 4-substituted acetophenone (**9a**,**b**) with different aldehydes **10a–f** at room temperature in methanol provided the corresponding chalcones **11a–l** in high yields (70–95 %). Cyclization of the chalcones **11a–l** with 4-hydrazinobenzenesulfonamide hydrochloride (**12**) in refluxing ethanol afforded the target pyrazole derivatives **13a–l** in good yields (75–95 %). 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 chalcone compounds **11k**,**l** showed sharp peak at 1656–1648 cm^{-1} corresponding to C=O while, the ^{1}H NMR spectra of **11 k**,**l** displayed two doublets, each of one proton intensity, one at δ 7.55–7.56 and the second at δ 7.85–7.91 with high *J* value (15.2 Hz) indicating the 2 olefinic protons. ^{13}C NMR spectra of **11k,l** confirmed also the presence of α, β-unsaturated carbonyl system of chalcones by presence of three peaks at δ 128.6–133.1, 129.6–140.2 and 188.4–188.5 corresponding to propenone C-3, propenone C-2 and C-1 of C=O respectively.

The IR spectra of dihydropyrazole compounds **13a–l** showed either a broad or forked peak at 3433–3260 cm^{-1} corresponding to NH_2 in addition to two sharp peaks, one at 1157–1149 cm^{-1} and the second at 1354–1323 cm^{-1} corresponding to SO_2 while, the ^{1}H NMR spectra of **13a-l** showed three signals as doublet of doublet (dd), each of one proton intensity, one at δ 3.13–3.43, second at δ 3.92–4.03, and the third at δ 5.57–6.16 corresponding to two protons at position 4 and one proton at position 5, respectively of the dihydropyrazole ring. Each proton has two *J* values, the two protons at position 4 have higher *J* value (17.6 Hz) indicating coupling between each other and lower *J* values indicating coupling of each one of them with the proton at position 5. Accordingly, proton at position 5 has two *J* values indicating it's coupling with the two protons at position 4. Additionally, ^{13}C NMR spectra of **13a–l** confirmed the presence of the dihydropyrazole ring due to presence of three peaks at δ 39.0–44.8, 56.2–63.4 and 148.0–163.0 corresponding to dihydropyrazole C-4, C-5 and C-3, respectively.

Biological evaluation

In vitro cyclooxygenase (COX) inhibition assay

In order to study the biological activity of the target compounds **13a–l**, we tested their ability to inhibit both ovine COX-1 and COX-2 isozymes. The efficacies of tested compounds were determined through using a concentration causing 50 % enzyme inhibition (IC_{50}) (Table 1). The results revealed that the potency of the tested compounds **13a–l** are ranging from potent to moderately potent of the inhibitory activity for COX-1 (IC_{50} = 3.6–19.8 μM) and COX-2 (IC_{50} = 0.84–6.8 μM). Out of the twelve dihydropyrazole series, **13f**, **13j**, **13l** and **13k** showed more inhibitory activity towards both COX-1 (IC_{50} = 3.6, 3.7, 4.2 and 5.1 μM, respectively) and COX-2 (IC_{50} = 0.84, 0.93, 0.98 and 1.15 μM, respectively) than celecoxib (IC_{50} = 9.7 and 1.33 μM for COX-1 and COX-2, respectively). Additionally, they had moderate COX-2 selectivity index (COX-2 SI = 4.28, 3.97, 4.28 and 4.43) compared to reference drug celecoxib (COX-2 SI = 7.2). On the other hand, the other eight tested compounds showed a moderate inhibitory activity against both COX-1 and COX-2 in the following order **13b** > **13i** > **13a** > **13e** > **13c** > **13d** > **13g** > **13h** compared to celecoxib.

Anti-inflammatory activity

Anti-inflammatory activity of compounds **13a–l** was evaluated by performing the standard formalin-induced foot paw edema method in rats using celecoxib as a reference drug (Turner 1965). ED_{50} of each compound determined after 3 h are depicted in (Table 2). ED_{50} of compounds **13f**, **13j**, **13k** and **13l** which showed the best

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

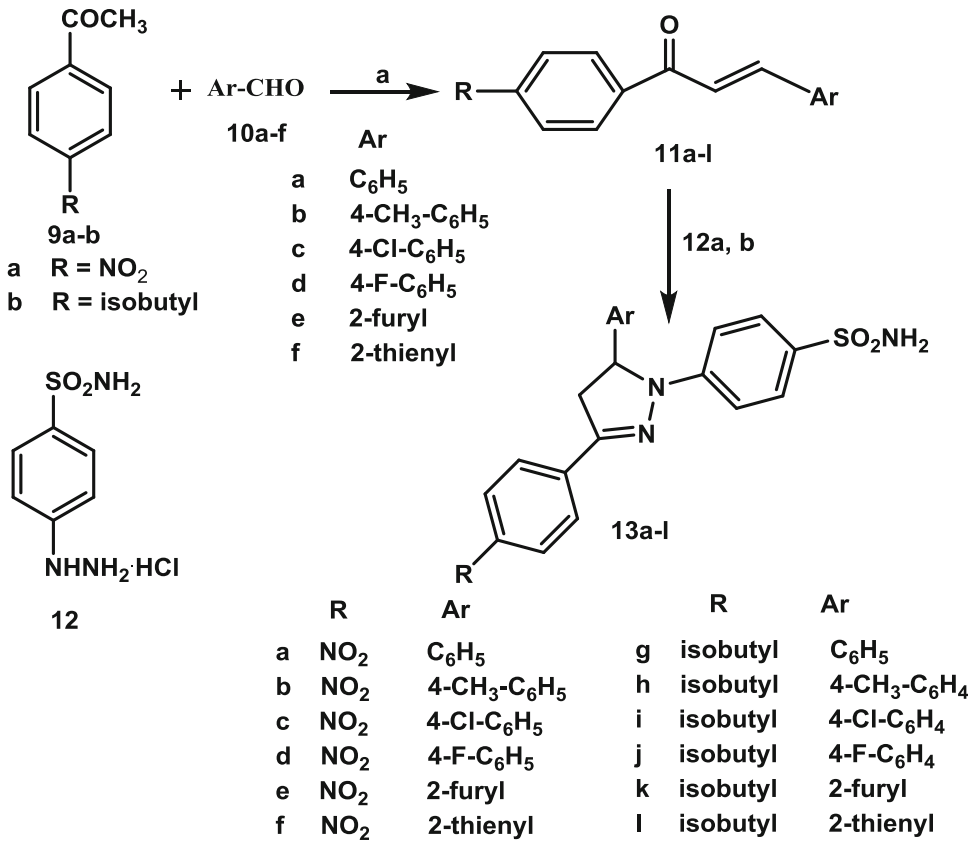

Table 1 In vitro COX-1 and COX-2 inhibition and anti-inflammatory activity of dihydropyrazoles **13a–l** and celecoxib

Compound no.	IC_{50} (μM)[a]		
	COX-1	COX-2	COX-2 selectivity index[b]
13a	12.4	3.4	3.64
13b	8.9	2.2	4.04
13c	14.6	4.6	3.17
13d	17.9	5.2	3.44
13e	13.8	3.8	3.63
13f	3.6	0.84	4.28
13g	19.8	5.9	3.35
13h	21.3	6.8	3.13
13i	10.9	2.8	3.89
13j	3.7	0.93	3.97
13k	5.1	1.15	4.43
13l	4.2	0.98	4.28
Celecoxib	9.7	1.33	7.29

[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] Selectivty index (COX-1 IC_{50}/COX-2 IC_{50})

cyclooxygenase activities indicated their weak anti-inflammatory activities (ED_{50} = 118, 68, 90 and 117 mg/kg, respectively) compared to celecoxib (ED_{50} = 18 mg/kg). While compounds **13i**, **13e** and **13h** showed moderate in vitro cyclooxygenase activities, their ED_{50} indicated that they had the best anti-inflammatory activities of all compounds (ED_{50} = 18, 32 and 36 mg/kg, respectively) compared to celecoxib (ED_{50} = 18 mg/kg).

Ulcerogenic liability

The ulcerogenic potential of compounds **13a–l** (50 mg/kg) was evaluated according to Meshali's method and the ulcer index was calculated according to Robert's method (Hassan et al. 2014).The ulcerogenic effect was compared to celecoxib (50 mg/kg). From the data obtained, (Table 3), it had been observed that compounds **13d**, **13l** and **13i** caused less gastric ulceration effect (ulcer index of compound **13d**: 2.7; **13l**: 2.7; **13i**: 2.8) in the experimental animals, compared to the standard drug celecoxib (ulcer index 3.35). Also, it had been noted that compounds **13b**, **13h**, **13e** and **13k** have a gastric safety profile very close to celecoxib (ulcer index of compound **13b**: 3.97; **13h**: 3.7; **13e**: 3.95; **13k**: 3.97).

Table 2 The anti-inflammatory activity (ED_{50}, mg/kg) of dihydropyrazoles **13a–l** and celecoxib after 3 h against formalin-induced paw edema in rats

Compound no.	ED_{50} (mg/kg) 3 h
13a	65
13b	45
13c	54
13d	54
13e	32
13f	118
13g	90
13h	36
13i	24
13j	68
13k	90
13l	117
Celecoxib	18

Conclusion

In summary, the present study reported the design and synthesis of novel celecoxib analogues. The synthesized compounds were evaluated for their COX-1/COX2 activity in vitro, anti-inflammatory activity in vivo and ulcerogenic liability. COX-1 and COX-2 studies declared that all compounds **13a–l** showed selectivity towards COX-2 more than COX-1 due to presence of SO_2NH_2 moiety which is essential for COX-2 selectivity. Structure activity relationship showed that in 4-nitrophenyl compounds **13a–f** the introduction of small hetero aryl group, furan **13e** or thiophene **13f**, in position 5 of pyrazoline ring showed anti-inflammatory activity ranging from weak (**13f** $ED_{50} = 118$ mg/kg) to moderate (**13e** $ED_{50} = 32$ mg/kg) compared to celecoxib ($ED_{50} = 18$ mg/kg) although in case of thiophene **13f**, it had COX-1 $IC_{50} = 3.6$ and COX-2 $IC_{50} = 0.84$ μM less than celecoxib ($IC_{50} = 0.87$ μM for COX-2 and 6.7 μM for COX-1). In addition, in 4-isobutylphenyl compounds **13g–l** the introduction of small hetero aryl group, furan **13k** or thiophene **13l**, in position 5 of pyrazoline ring decreases the anti-inflammatory activity ($ED_{50} = 90$, 117 mg/kg for **13k** and **13l**, respectively) compared to celecoxib ($ED_{50} = 18$ mg/kg) although they both had COX-1 $IC_{50} = 5.1$, 4.2 μM respectively and COX-2 $IC_{50} = 1.15$, 0.98 μM, respectively less than celecoxib ($IC_{50} = 0.87$ μM for COX-2 and 6.7 μM for COX-1). In addition, compound **13 l** has an improved gastric safety profile (**13l** ulcer index = 2.7) compared to celecoxib (ulcer index = 3.35). Moreover, in case of 4-isobutylphenyl compounds **13g–l** the introduction of 4-fluorophenyl **13h** or 4-chlorophenyl **13i** exhibited anti-inflammatory activity ranging from moderate (**13h** $ED_{50} = 36$ mg/kg) to strong (**13i** $ED_{50} = 24$ mg/kg) compared to celecoxib ($ED_{50} = 18$ mg/kg) with gastric safety profile ranging from comparable (**13h** ulcer index = 3.7) to better (**13i** ulcer index = 2.8) than celecoxib (ulcer index = 3.35). In conclusion, in our trials to synthesize celecoxib like compounds the in vitro and in vivo structure activity relationship acquired suggest that the replacement of pyrazole, trifluoromethyl and tolyl group in celecoxib with dihydropyrazole, 4-isobutylphenyl and 4-chlorophenyl respectively in compound **13i** almost retain the anti-inflammatory activity compared to celecoxib but with improved gastric safety profile and moderate COX-2 selectivity index compared to celecoxib. Consequently, the previous compounds provide us with new scaffolds for the development of novel compounds having in vivo anti-inflammatory activity comparable to celecoxib but with improved gastric safety profile.

Table 3 Ulcerogenic effect of dihydropyrazoles **13a–l** and celecoxib in rats (50 mg/kg)

Compound	Average severity	Average no of ulcers	% Incidence/10	Ulcer index
13a	0.67 ± 0.013	0.4 ± 0.005	3	4.07
13b	0.67 ± 0.013	0.3 ± 0.004	3	3.97
13c	0.62 ± 0.012	0.4 ± 0.005	4	5.02
13d	0.5 ± 0.007	0.2 ± 0.003	2	2.7
13e	0.75 ± 0.024	0.2 ± 0.004	3	3.95
13f	0.5 ± 0.003	0.4 ± 0.003	4	4.9
13g	0.75 ± 0.0014	0.3 ± 0.006	3	4.05
13h	0. 0.5 ± 0.002	0.2 ± 0.005	3	3.7
13i	0.5 ± 0.004	0.3 ± 0.008	2	2.8
13j	0.83 ± 0.004	0.4 ± 0.002	4	5.23
13k	0.67 ± 0.008	0.3 ± 0.001	3	3.97
13l	0.5 ± 0.008	0.2 ± 0.001	2	2.7
Celecoxib	0.63 ± 0.022	0.72 ± 0.016	2	3.35
DMSO	0	0	0	0

References

Abdelazeem, A.H., S.A. Abdelatef, M.T. El-Saadi, H.A. Omar, S.I. Khan, C.R. McCurdy, and S.M. El-Moghazy. 2014. Novel pyrazolopyrimidine derivatives targeting COXs and iNOS enzymes; design, synthesis and biological evaluation as potential anti-inflammatory agents. *European Journal of Pharmaceutical Sciences* 62: 197–211.

Abdellatif, K.R.A., M.A. Chowdhury, and E.E. Knaus. 2008a. Synthesis of new 1-[4-methane (amino) sulfonylphenyl)]-5-[4-(aminophenyl)]-3-trifluoromethyl-1*H*-pyrazoles. *Journal of Heterocyclic Chemistry* 45: 1707–1710.

Abdellatif, K.R.A., M.A. Chowdhury, C.A. Velázquez, Z. Huang, Y. Dong, D. Das, G. Yu, M.R. Suresh, and E.E. Knaus. 2010. Celecoxib prodrugs possessing a diazen-1-ium-1,2-diolate nitric oxide donor moiety: synthesis, biological evaluation and nitric oxide release studies. *Bioorganic & Medicinal Chemistry Letters* 20: 4544–4549.

Abdellatif, K.R.A., M.A. Chowdhury, Y. Dong, D. Das, G. Yu, C. Velázquez, M.R. Suresh, and E.E. Knaus. 2009. Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 5-(4-carboxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole and its aminosulfonyl analog: Synthesis, biological evaluation and nitric oxide release studies. *Bioorganic & medicinal chemistry* 17: 5182–5188.

Abdellatif, K.R.A., M.A. Chowdhury, Y. Dong, and E.E. Knaus. 2008b. Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids: synthesis, nitric oxide release studies and anti-inflammatory activities. *Bioorganic & medicinal chemistry* 16: 6528–6534.

Abdellatif, K.R.A., M.A. Chowdhury, Y. Dong, C. Velázquez, D. Das, M.R. Suresh, and E.E. Knaus. 2008c. Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole and its methanesulfonyl analog: synthesis, biological evaluation and nitric oxide release studies. *Bioorganic & Medicinal Chemistry* 16: 9694–9698.

Ahlström, M.M., M. Redderström, I. Zamora, and K. Luthman. 2007. CYP2C9 structure–metabolism relationships: Optimizing the metabolic stability of COX-2 inhibitors. *Journal of Medicinal Chemistry* 50: 4444–4452.

Ahn, J.H., H.M. Kim, S.H. Jung, S.K. Kang, K.R. Kim, S.D. Rhee, S.D. Yang, H.G. Cheon, and S.S. Kim. 2004. Synthesis and DP-IV inhibition of cyano-pyrazoline derivatives as potent anti-diabetic agents. *Bioorganic & Medicinal Chemistry Letters* 14: 4461–4465.

Al-Hourani, B.J., S.K. Sharma, J.Y. Mane, J. Tuszynski, V. Baracos, T. Kniess, M. Suresh, J. Pietzsch, and F. Wuest. 2011. Synthesis and evaluation of 1,5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors. *Bioorganic & Medicinal Chemistry Letters* 21: 1823–1826.

Amutha, P., and S. Nagarajan. 2012. Synthesis and antimicrobial activities of new 4,6-diaryl-4,5-dihydro-3-hydroxy-2*H*-indazoles. *Journal of Heterocyclic Chemistry* 49: 428–432.

Bano, S., K. Javed, S. Ahmad, I.G. Rathish, S. Singh, and M.S. Alam. 2011. Synthesis and biological evaluation of some new 2-pyrazolines bearing benzene sulfonamide moiety as potential anti-inflammatory and anti-cancer agents. *European Journal of Medicinal Chemistry* 46: 5763–5768.

Bashir, R., S. Ovais, S. Yaseen, H. Hamid, M.S. Alam, M. Samim, S. Singh, and K. Javed. 2011. Synthesis of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide as anti-cancer and anti-inflammatory agents. *Bioorganic & Medicinal Chemistry Letters* 21: 4301–4305.

Chandra, T., N. Garg, S. Lata, K.K. Saxena, and A. Kumar. 2010. Synthesis of substituted acridinyl pyrazoline derivatives and their evaluation for anti-inflammatory activity. *European Journal of Medicinal Chemistry* 45: 1772–1776.

Chowdhury, M.A., K.R.A. Abdellatif, Y. Dong, D. Das, M.R. Suresh, and E.E. Knaus. 2008a. Synthesis of celecoxib analogs that possess a N-hydroxypyrid-2(1H)one 5-lipoxygenase pharmacophore: biological evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity. *Bioorganic & Medicinal Chemistry Letters* 18: 6138–6141.

Chowdhury, M.A., K.R.A. Abdellatif, Y. Dong, and E.E. Knaus. 2008b. Synthesis of new 4-[2-(4-methyl(amino)sulfonylphenyl)-5-trifluoromethyl-2*H*-pyrazol-3-yl]-1,2,3,6-tetrahydropyridines: A search for novel nitric oxide donor anti-inflammatory agents. *Bioorganic & Medicinal Chemistry* 16: 8882–8888.

Dogné, J.M., C.T. Supuran, and D. Pratico. 2005. Adverse cardiovascular effects of the coxibs. *Journal of Medicinal Chemistry* 48: 2251–2257.

Fioravanti, R., A. Bolasco, F. Manna, F. Rossi, F. Orallo, F. Ortuso, S. Alcaro, and R. Cirilli. 2010. Synthesis and biological evaluation of *N*-substituted-3,5-diphenyl-2-pyrazoline derivatives as cyclooxygenase (COX-2) inhibitors. *European Journal of Medicinal Chemistry* 45: 6135–6138.

Fun, H.K., S. Chantrapromma, P.S. Patil, E.D. D'Silva, and S.M. Dharmaprakash. 2008. (E)-3-(4-methyl-phen-yl)-1-(4-nitro-phenyl)prop-2-en-1-one. *Acta Crystallographica Section E, Structure Reports Online* 64: o954–o955.

Gupta, R.A., and S.G. Kaskhedikar. 2012. Synthesis, antitubercular activity, and QSAR analysis of substituted nitroaryl analogs: Chalcone, pyrazole, isoxazole, and pyrimidines. *Medicinal Chemistry Research* 22: 3863–3880.

Habeeb, A.G., P.N.P. Rao, and E.E. Knaus. 2001. Design and synthesis of celecoxib and rofecoxib analogues as selective cyclooxygenase-2 (COX-2) Inhibitors: Replacement of sulfonamide and methylsulfonyl pharmacophores by an azido bioisostere. *Journal of Medicinal Chemistry* 44: 3039–3042.

Hassan, G.S., S.M. Abou-Seri, G. Kamel, and M.M. Ali. 2014. Celecoxib analogs bearing benzofuran moiety as cyclooxygenase-2 inhibitors: Design, synthesis and evaluation as potential anti-inflammatory agents. *European Journal of Medicinal Chemistry* 76: 482–493.

Johnson, M., B.Younglove, L. Lee, R. LeBlanc, H.H. Jr., P. Hills, H. Mackay, T. Brown, S.L. Mooberry, and M. Lee. 2007. Design, synthesis, and biological testing of pyrazoline derivatives of combretastatin-A4. *Bioorganic & medicinal chemistry letters* 17: 5897–901.

Khode, S., V. Maddi, P. Aragade, M. Palkar, P.K. Ronad, S. Mamledesai, A.H.M. Thippeswamy, and D. Satyanarayana. 2009. Synthesis and pharmacological evaluation of a novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents. *European Journal of Medicinal Chemistry* 44: 1682–1688.

Lange, J.H.M., H.H.V. Stuivenberg, W. Veerman, H.C. Wals, B. Stork, H.K.A.C. Coolen, A.C. McCreary, T.J.P. Adolfs, and C.G. Kruse. 2005. Novel 3,4-diarylpyrazolines as potent cannabinoid CB1 receptor antagonists with lower lipophilicity. *Bioorganic & Medicinal Chemistry Letters* 15: 4794–4798.

Ovais, S., R. Bashir, S. Yaseen, P. Rathore, M. Samim, and K. Javed. 2012. Synthesis and pharmacological evaluation of some novel 2-pyrazolines bearing benzenesulfonamide as anti-inflammatory and blood glucose lowering agents. *Medicinal Chemistry Research* 22: 1378–1385.

Ovais, S., S. Yaseen, R. Bashir, P. Rathore, M. Samim, S. Singh, V. Nair, and K. Javed. 2013. Synthesis and anti-inflammatory activity of celecoxib like compounds. *Journal of Enzyme Inhibition and Medicinal Chemistry* 28: 1105–1112.

Rathish, I.G., K. Javed, S. Ahmad, S. Bano, M.S. Alam, K.K. Pillai, S. Singh, and V. Bagchi. 2009. Synthesis and antiinflammatory activity of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide. *Bioorganic & Medicinal Chemistry Letters* 19: 255–258.

Romagnoli, R., P.G. Baraldi, M.D. Carrion, O. Cruz-Lopez, C.L. Cara, J. Balzarini, E. Hamel, A. Canella, E. Fabbri, R. Gambari, G. Basso, and G. Viola. 2009. Hybrid alpha-bromoacryloylamido chalcones. Design, synthesis and biological evaluation. *Bioorganic & Medicinal Chemistry Letters* 19: 2022–2028.

Rostom, S.A.F. 2006. Synthesis and in vitro antitumor evaluation of some indeno[1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems. *Bioorganic & Medicinal Chemistry* 14: 6475–6485.

Sahu, S.K., M. Banerjee, A. Samantray, C. Behera, and M.A. Azam. 2008. Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel pyrazoline derivatives. *Tropical Journal of Pharmaceutical Research* 7: 961–968.

Scheen, A.J. 2004. Withdrawal of rofecoxib (Vioxx): what about cardiovascular safety of COX-2 selective non-steroidal anti-inflammatory drugs. *Revue Medicale de Liege* 59: 565–569.

Turner, R.A. 1965. *Screening method in pharmacology. Anti-inflammatory agent 13*, 158. New York: Academic Press.

Zarghi, A., L. Najafnia, B. Daraee, O.G. Dadrass, and M. Hedayati. 2007. Synthesis of 2,3-diaryl-1,3-thiazolidine-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. *Bioorganic & Medicinal Chemistry Letters* 17: 5634–5637.

Zarghi, A., T. Zebardast, B. Daraie, and M. Hedayati. 2009. Design and synthesis of new 1,3-benzthiazinan-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. *Bioorganic & Medicinal Chemistry* 17: 5369–5373.

Zebardast, T., A. Zarghi, B. Daraie, M. Hedayati, and O.G. Dadrass. 2009. Design and synthesis of 3-alkyl-2-aryl-1,3-thiazinan-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. *Bioorganic & Medicinal Chemistry Letters* 19: 3162–3165.

Zheng, C.J., S.M. Jiang, Z.H. Chen, B.J. Ye, and H.R. Piao. 2011. Synthesis and anti-bacterial activity of some heterocyclic chalcone derivatives bearing thiofuran, furan, and quinoline moieties. *Archiv der Pharmazie* 344: 689–695.