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SYNTHESIS AND ANTI-INFLAMMATORY EVALUATION OF NEW SUBSTITUTED 1-(3-CHLOROPHENYL)-3-(4-METHOXYPHENYL)-1*H*-PYRAZOLE DERIVATIVES

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Abstract: A series of heterocyclic derivatives including 1,2,4-triazole-3(4H)-one (**3a,b**), 1H-pyrazol-5(4H)-one (**4,5**), 1H-pyrazol-4-carbonitrile (**7**), pyridine-3-carbonitrile (**8, 9a,b**), pyrimidine-5-carbonitrile (**10a,b**), methylpyrimidin-2(1H)-one or thione (**11a,b**), pyrimidine-5-carboxylate (**12a,b**), quinazolin-5(6H)-one (**13a,b**) and indeno [1,2-d] pyrimidin-5-one (**14a,b**) moieties conjugated with 1,3-disubstituted pyrazole moiety were synthesized on reaction with semicarbazide, thiosemicarbazide, 3-amino-5-oxo-2-pyrazoline, cyanoacetohydrazide, 2-acetyl thiophene, p-chloroacetophenone, urea, thiourea and 1,3-dicarbonyl compounds, respectively, by using 1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazole-4-carboxaldehyde (**2**) as starting material. The structures of all the newly synthesized products have been established on the basis of analytical and spectral data. The anti-inflammatory screening showed that most of the obtained compounds were found to have significant anti-inflammatory activities with prostaglandin inhibition at a dose level of 2.5 and 5 mg/kg comparable to celecoxib as a reference control. The ulcer indices of all compounds are mainly in the safe level (UI = 2.10–4.27) except for compounds **9a** and **14a**, which were highly ulcerogenic.

Keywords: pyrazole derivatives, anti-inflammatory, selective COX-2 inhibitor

Prostaglandins are endogenous substances involved in different processes of physiological nature and are potent mediators of inflammation. Prostaglandins are produced, together with other prostanoids, in the arachidonic acid metabolism, whose first step, consisting of the oxidative conversion of arachidonic acid into prostaglandin H_2, is catalyzed by cyclooxygenase (COX) (1). This enzyme exists at least as two isoforms, one constitutive (COX-1) and the other inducible (COX-2) (2). COX-1 is found in platelets, kidneys, and in the gastrointestinal (GI) tract, and is believed to be responsible for the homeostatic maintenance of the kidneys and GI tract. The COX-2 enzyme is the inducible isoform that is produced by various cell types upon exposure to cytokines, mitogens and endotoxins released during injury (3). The COX-2 enzyme, after being overexpressed at the site of injury, is a catalyst for the production of the prostaglandins that results in inflammation and pain at the site. COX-1 is involved in the maintenance of the GI tract; so all nonsteroidal anti-inflammatory drugs (NSAIDs) that are inhibitors of both COX-2 and COX-1 have been found to cause side effects associated with gastrointestinal ulcers (4–6). Thus, it was thought that a more selective COX-2 inhibitor would have reduced gastrointestinal side effects (3). Several COX-2 selective inhibitors, including celecoxib (Celebrex) (7), valdecoxib (Bextra) (8), rofecoxib (Vioxx) (9), and etoricoxib (Arroxin) (10) have shown excellent efficacy in humans with few side effects. There is still a need to synthesize novel, potent analgesic/anti-inflammatory compounds that have reduced side effects when compared with the drugs currently available on the market. In recent decades, the literature has been enriched with progressive findings about the synthesis and pharmacological activities of pyrazole ring, which is a core structure

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in various synthetic pharmaceuticals displaying a wide variety of biological activities as anti-inflammatory (11–14) and analgesic (15–17). They have also been reported as phosphodiesterase 4 (PDE4) inhibitors in immune and inflammatory cells (18). So, based on these findings, we prepared in this context, a series of novel pyrazole derivatives containing pyrazole, pyrimidine and thiazolopyrimidine rings as substituents. Our objective from synthesis of these pyrazole derivatives is to study the effect of introducing heterocyclic derivatives on the pharmacological activities of target compounds.

EXPERIMENTAL

Chemistry

Melting points were measured in open capillary tubes using Griffin apparatus and were uncorrected. The infra red (IR) spectra were recorded using potassium bromide disc technique on Shimadzu 435 IR Spectrophotometer at Microanalytical unit, National Research Centre (NRC). The proton nuclear magnetic resonance (^{1}H-NMR) spectra were performed on Varian Gemini 300 MHz spectrophotometer using tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) are given using parts per million scale (ppm). Mass spectra were recorded on Hewlett Packard 5988 spectrometer at Microanalytical unit (NRC). Elemental microanalyses were carried out at Microanalytical unit (NRC). All reactions were monitored by TLC using precoated aluminum sheets with silica gel Merck 60 F 254 and were visualized by UV lamp. Synthetic routes are presented in Schemes 1,2. Chemical naming, calculation of molecular weight (m.w.) and microanalyses of new compounds 2-14 were performed using ChemDraw Program.

1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carboxaldehyde (2)

A mixture of 4-methoxyacetophenone (15 g, 0.1 mol), 3-chlorophenylhydrazine hydrochloride (17.9 g, 0.1 mol) and crystalline sodium acetate (8.2 g, 0.1 mol) in absolute ethanol (200 mL) was refluxed for 6 h. After cooling and dilution with water, the formed precipitate was filtered, washed with water and air dried to give compound **1** with nearly quantitative yield. It was introduced to the next step without further purification. Phosphorus oxychloride (20 mL), was added to N,N-dimethylformamide (150 mL) at 0°C and stirred for 30 min. Compound 1 (13.5 g, 0.049 mol) was added portionwise to this mixture and stirred for 15 h. The crude reaction mixture was then quenched into one liter of water and stirred for additional 15 h. The resulting solid was filtered and then dissolved in methylene chloride and dried over magnesium sulfate, evaporated till dryness and then crystallized from ether/hexane (5:1, v/v). Yield 84%; m.p. 98–100°C; IR (KBr, cm^{-1}): 1705 (C=O), 1653 (C=N), 1620 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.88 (s, 3H, OCH_3), 7.05–8.10 (m, 8H, Ar-H), 9.48 (s, 1H, CH of pyrazole), 10.00 (s, 1H, CHO). MS (m/z, %): 314 (37.8, M^+ + 2), 312 (100, M^+), 285 (3.8), 283 (13.1), 207 (2.5), 205 (6.1), 201 (2.7), 113 (9.7), 111 (26.4), 75 (30.4). Analysis: calc. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96%; found: C, 65.55; H, 4.20; N, 8.92%.

5-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-2H-1,2,4-triazole-3(4H)-one (3a)

A mixture of compound **2** (0.31 g, 0.001 mol), semicarbazide hydrochloride (0.11 g, 0.001 mol) and crystalline sodium acetate (0.14 g, 0.001 mol) in absolute ethanol (30 mL) was refluxed for 3 h. After cooling and dilution with water, the product formed was filtered, washed with water, air dried and crystallized from absolute ethanol. Yield 69%; m.p. 180–182°C; IR (KBr, cm^{-1}): 3495, 3379 (2 NH), 1683 (C=O), 1645 (C=N), 1594 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.81 (s, 1H, OCH_3), 6.48 (s, 1H, NH exchangeable with D_2O), 7.04–7.97 (m, 8H, Ar-H), 9.11 (s, 1H, CH of pyrazole), 10.14 (s, 1H, NH exchangeable with D_2O). MS (m/z, %): 370 (2.7, M^+ + 3), 368 (8.3, M^+ + 1), 311 (39.4), 309 (100.0), 296 (7.3), 294 (20.4), 268 (14.2), 266 (40.6), 113 (24.1), 111 (65.8), 75 (56.3), 58 (31.6). Analysis: calc. for $C_{18}H_{14}ClN_5O_2$: C, 58.78; H, 3.84; N, 19.04%; found: C, 58.60; H, 3.85; N, 19.11%.

5(1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-2H-1,2,4-triazole-3(4H)-thione (3b)

A mixture of compound **2** (0.31 g, 0.001 mol) and thiosemicarbazide (0.91 g, 0.01mol) in absolute ethanol (15 mL) containing few drops of glacial acetic acid was refluxed for 2 h. After cooling and dilution with water, the solid formed was filtered, washed with water, air dried and crystallized from absolute ethanol. Yield 72%; m.p. 185–187°C; IR (KBr, cm^{-1}): 3327, 3151 (2 NH), 1646 (C=N), 1594 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.81 (s, 3H, OCH_3), 7.04–8.18 (m, 8H, Ar-H), 8.30 (s, 1H, NH exchangeable with D_2O), 9.19 (s, 1H, CH of pyrazole), 11.36 (s, 1H, NH exchangeable with D_2O). MS (m/z, %): 386 (2.1, M^+ + 3), 384 (6.0, M^+ + 1), 311 (24.3), 309 (65.0), 310 (67.9), 308 (100.0), 113 (30.6), 111 (83.5), 107 (2.2), 75 (72.8), 74

(22.3).Analysis: calc. for $C_{18}H_{14}ClN_5OS$: C, 56.32; H, 3.68; N, 18.24%; found: C, 56.48; H, 3.66; N, 18.31%.

(4E)-3-amino-4-[(1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]methylene)-1H-pyrazol-5(4H)-one (4)

A mixture of compound **2** (0.31 g, 0.001 mol) and 3-amino-5-oxopyrazoline (0.1 g, 0.001 mol) in absolute ethanol (15 mL) containing few drops of triethylamine was refluxed for 1 h. After cooling, the solid formed was filtered, washed with cold ethanol, air dried and crystallized from ethanol. Yield 82%; m.p. 230–232°C; IR (KBr, cm^{-1}): 3411 [(NH, NH_2) br.], 1684 (C=O), 1661 (C=N), 1592 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.81 (s, 3H, OCH_3), 6.90–8.14 (m, 8H, Ar-H), 8.53 (CH=C of 3-aminopyrazolone), 9.07 (s, 2H, NH_2 exchangeable with D_2O), 9.25 (s, 1H, CH of pyrazole), 11.76 (s, 1H, NH exchangeable with D_2O). Analysis: calc. for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78%; found: C, 61.11; H, 4.11; N, 17.73%.

(3E)-3-([1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]methyleneamino-1H-pyrazol-5(4H)-one (5)

A mixture of compound **2** (0.31 g, 0.001 mol) and 3-amino-5-oxopyrazoline (0.1 g, 0.001 mol) in absolute ethanol (15 mL) containing few drops of glacial acetic acid was refluxed for 1 h. After cooling, the solid formed was filtered, washed with cold ethanol, air dried and crystallized from ethanol. Yield 76%; m.p. 204–206°C; IR (KBr, cm^{-1}): 3448 (NH), 1676 (C=O), 1648 (C=N), 1595 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.81 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2 of pyrazolinone), 7.05–8.20 (m, 8H, Ar-H), 8.70 (s, 1H, CH=N), 9.06 (s, 1H, CH of pyrazole), 11.60 (s, 1H, NH exchangeable with D_2O). MS (m/z, %): 395 (7.6, M^+ + 2), 393 (21.4, M^+), 311 (39.5), 309 (100.0), 297 (2.7), 295 (7.8), 113 (13.3), 111 (40.6), 75 (33.0). Analysis: calc. for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78%; found: C, 60.75; H, 4.10; N, 17.73%.

(E)-3-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]–2-cyanoacrylohydrazide (6)

A mixture of compound **2** (0.31 g, 0.001 mol) and cyanoacetohydrazide (0.1 g, 0.001 mol) in ethanol (15 mL) containing few drops of triethylamine was stirred vigorously for 3 h at room temperature then left overnight. The precipitate formed was filtered, washed with cold ethanol, air dried and crystallized from ethanol. Yield 72%; m.p. 205–207°C; IR (KBr, cm^{-1}): 3444, 3132 (NH_2, NH), 2227 (C≡N), 1716 (C=O), 1647 (C=N), 1595 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.80 (s, 3H, OCH_3), 7.01–8.10 (m, 8H, Ar-H), 8.40 (br. s, 2H, NH_2 exchangeable with D_2O), 8.81 (s, 1H, CH=C), 9.14 (s, 1H, CH of pyrazole), 11.53 (s, 1H, NH exchangeable with D_2O). MS (m/z, %): 395 (25.4, M^+ + 2), 393 (67.8, M^+), 312 (13.2), 310 (33.1), 311 (42.2), 309 (100.0), 113 (21.3), 111 (61.6), 75 (38.0). Analysis: calc. for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78%; found: C, 61.11; H, 4.10; N, 17.74%.

3-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-4,5-dihydro-5-oxo-1H-pyrazol-4-carbonitrile (7)

Method A: Compound **6** (0.4 g, 0.001 mol) was heated under reflux in acetic acid (85%, 10 mL) for 3 h. The reaction mixture was then partially evaporated and left to precipitate. The product obtained was filtered, washed with water, air dried and crystallized from ethanol; yield 60%.

Method B: a mixture of compound **2** (0.31 g, 0.001 mol) and cyanoacetohydrazide (0.1 g, 0.001 mol) in ethanol (20 mL) containing few drops of glacial acetic acid was refluxed for 2–3 h, and left to cool. The solid formed was filtered, washed with cold ethanol, air dried and crystallized from ethanol; yield 80%.

M.p. 227–230°C; IR (KBr, cm^{-1}): 3417 (NH), 2208 (C≡N), 1681 (C=O), 1643 (C=N), 1591 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.81 (s, 3H, OCH_3), 4.12 (s, 1H, CH-CN), 7.05–8.20 (m, 8H, Ar-H), 9.06 (s, 1H, CH of pyrazole), 11.63 (s, 1H, NH exchangeable with D_2O). Analysis: calc. for $C_{20}H_{14}ClN_5O_2$: C, 61.31; H, 3.60; N, 17.87%; found: C, 61.12; H, 3.61; N, 17.94%.

2-Amino-4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-6-(thien-2-yl)pyridine-3-carbonitrile (8)

A mixture of compound **2** (3.1 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and ammonium acetate (0.77 g, 0.01 mol) in ethanol (30 mL) was added to 2-acetylthiophene (1.3 mL, 0.01 mol). The reaction mixture was refluxed for 3–4 h. After cooling, the solid formed was filtered, washed with water, air dried and crystallized from benzene. Yield 65%; m.p 217–220°C; IR (KBr, cm^{-1}): 3417, 3307 (NH_2), 2208 (C≡N), 1681 (C=N), 1591 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.27 (s, 2H, NH_2 exchangeable with D_2O), 3.81 (s, 3H, OCH_3), 7.01–8.18 (m, 11H, Ar-H and thiophene protons), 9.26 (s, 1H, CH of pyrazole), 9.54 (s, 1H, CH of pyridine). MS (m/z, %): 485 (0.04, M^+ + 2), 483 (0.3, M^+), 469 (42.8), 467 (100.0), 444 (0.6), 442

(1.6), 310 (12.1), 308 (33.2), 113 (14.6), 111 (42.5), 75 (21.6). Analysis: calc. for $C_{26}H_{18}ClN_5OS$: C, 64.52; H, 3.75; N, 14.47%; found: C, 64.32; H, 3.76; N, 14.41%.

6-Aryl-4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2-dihydro-2-oxo- pyridine-3-carbonitrile (9a,b). General method

A solution of compound **2** (3.12 g, 0.01 mol), ethyl cyanoacetate (1.1 mL, 0.01 mol) and ammonium acetate (0.8 g, 0.01 mol) in ethanol (30 mL) was added to 2-acetylthiophene or 4-chloroacetophenone (0.01 mol). The reaction mixture was refluxed for 3–4 h. After cooling, the solid formed was filtered, washed with water, air dried and crystallized from benzene.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2-dihydro-2-oxo-6-(thien-2-yl) pyridine-3-carbonitrile (9a)

Yield 72%; m.p. 310–312°C; IR (KBr, cm^{-1}): 3153 (NH), 2214 (C≡N), 1707 (C=O), 1650 (C=N), 1595 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.10 (s, 1H, NH exchangeable with D_2O), 3.80 (s, 3H, OCH_3), 6.81 (s, 1H, CH of pyridone), 6.99–8.06 (m, 11H, Ar-H and thiophene protons), 9.06 (s, 1H, CH of pyrazole). MS (m/z, %): 486 (2.5, $M^+ + 2$), 484 (4.8, M^+), 201 (0.2), 170 (2.7), 113 (1.5), 111 (2.8), 108 (0.9), 107 (1.4), 106 (9.7), 105 (100.0), 77 (80.2), 75 (8.1). Analysis: calc. for $C_{26}H_{17}ClN_4O_2S$: C, 64.39; H, 3.53; N, 11.55%; found: C, 64.51; H, 3.52; N, 11.59%.

6-(4-Chlorophenyl)-4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2-dihydro-2-oxopyridine-3-carbonitrile (9b)

Yield 69%; m.p. > 300°C; IR (KBr, cm^{-1}): 3428 (NH), 2224 (C≡N), 1715 (C=O), 1639 (C=N), 1592 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.49 (s, 1H, NH exchangeable with D_2O), 3.78 (s, 3H, OCH_3), 6.71 (s, 1H, CH of pyridone), 7.00–8.05 (m, 12H, Ar-H), 9.05 (s, 1H, CH of pyrazole). Analysis: calc. for $C_{28}H_{18}Cl_2N_4O_2$: C, 65.51; H, 3.53; N, 10.91%; found: C, 65.25; H, 3.54; N, 10.89%.

6-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-2,4-dioxo or 4-oxo-2-thioxopyrimidine-5-carbonitrile (10a,b).

General method

A mixture of compound **2** (3.12 g, 0.01 mol), ethyl cyanoacetate (1.1 mL, 0.01 mol) and urea or thiourea (0.01 mol) in sodium ethoxide (0.5 g of sodium in 20 mL of ethanol) was stirred at room temperature overnight. The reaction mixture was poured onto hydrochloric acid-ice. The solid formed was filtered, washed with water, air dried and crystallized from methanol.

6-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-5-carbonitrile (10a)

Yield 78%; m.p. 240–242°C; IR (KBr, cm^{-1}): 3443, 3116 (2 NH), 2228 (C≡N), 1707, 1689 (2 C=O), 1646 (C=N), 1592 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.05 (s, 1H, NH exchangeable with D_2O), 3.83 (s, 3H, OCH_3), 3.97 (s, 1H, NH exchangeable with D_2O), 7.10–8.04 (m, 8H, Ar-H), 9.18 (s, 1H, CH of pyrazole). MS (m/z, %): 421 (0.4, $M^+ + 2$), 419 (0.9, M^+), 336 (49.4), 334 (100.0), 322 (2.5), 320 (6.3), 311 (5.3), 309 (13.0), 136 (1.2), 113 (28.6), 111 (72.7), 107 (2.7), 75 (50.3). Analysis: calc. for $C_{21}H_{14}ClN_5O_3$: C, 60.08; H, 3.36; N, 16.68%; found: C, 59.96; H, 3.37; N, 16.74%.

6-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-4-oxo-2-thioxopyrimidine-5-carbonitrile (10b)

Yield 75%; m.p. 223–225°C; IR (KBr, cm^{-1}): 3334, 3129 (2 NH), 2225 (C≡N), 1707 (C=O), 1689 (C=N), 1586 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.80 (s, 3H, OCH_3), 7.01–8.52 (m, 8H, Ar-H), 7.97 (s, 2H, 2 NH exchangeable with D_2O), 9.12 (s, 1H, CH of pyrazole). MS (m/z, %): 438 (2.8, $M^+ + 3$), 436 (20.2, $M^+ + 1$), 336 (12.4), 334 (26.1), 153 (2.9), 113 (13.8), 111 (33.3), 88 (2.1), 75 (23.2), 62 (100.0), 60 (21.9). Analysis: calc. for $C_{21}H_{14}ClN_5O_2S$: C, 57.86; H, 3.24; N, 16.07%; found: C, 58.09; H, 3.25; N, 16.01%.

5-Acetyl-4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-3,4-dihydro-6-methylpyrimidin-2(1H)-one or thione (11a,b). General method

A mixture of compound **2** (0.31 g, 0.001 mol), urea or thiourea (0.001 mol) and acetylacetone (0.1 mL, 0.001 mol) in absolute ethanol (20 mL) containing few drops of hydrochloric acid was refluxed for 4–6 h. After cooling, the solid formed was filtered, washed with water, air dried and crystallized from ethanol.

5-Acetyl-4-(1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3,4-dihydro-6-methylpyrimidine-2(1H)-one (11a)

Yield 62%; m.p. 148–150°C; IR (KBr, cm^{-1}): 3411, 3338 (2 NH), 1693, 1661 (2 C=O), 1651 (C=N), 1591 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.95 (s, 3H, CH_3), 2.27 (s, 3H, $COCH_3$), 2.71 (br.s,

2H, 2 NH exchangeable with D_2O), 3.81 (s, 3H, OCH_3), 5.41 (s, 1H, CH of pyrimidine), 6.86–8.36 (m, 8H, Ar-H), 9.16 (s, 1H, CH of pyrazole). MS (m/z, %): 439 (11.2, $M^+ + 3$), 437 (79.2, $M^+ + 1$), 424 (10.2), 422 (26.8), 287 (36.7), 285 (100.0), 153 (14.2), 113 (13.7), 111 (35.8), 75 (22.7). Analysis: calc. for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; N, 12.82%; found: C, 63.29; H, 4.83; N, 12.85%.

5-Acetyl-4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-3,4-dihydro-6 methylpyrimidine-2(1H)-thione (11b)

Yield 67%; m.p. 155–157°C; IR (KBr, cm^{-1}): 3398 (NH, br.), 1681 (C=O), 1646 (C=N), 1593 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.00 (s, 3H, CH_3), 2.30 (s, 2H, 2 NH exchangeable with D_2O), 2.85 (s, 3H, $COCH_3$), 3.81 (s, 3H, OCH_3), 5.4 (s, 1H, CH of pyrimidine), 6.91–8.01 (m, 8H, Ar-H), 9.22 (s, 1H, CH of pyrazole). Analysis: calc. for $C_{23}H_{21}ClN_4O_2S$: C, 60.99; H, 4.67; N, 12.37%. found: C, 61.17; H, 4.65; N, 12.33%.

Ethyl 4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-1,2,3,4-tetrahydro-6-methyl-2-oxo or thioxopyrimidine]-5-carboxylate (12a,b). General method

A mixture of compound **2** (0.31 g, 0.001 mol), urea or thiourea (0.001 mol) and ethyl acetoacetate (0.13 mL, 0.001 mol) in ethanol (20 mL) containing few drops of hydrochloric acid was refluxed for 5 h. After cooling, the solid formed was filtered, washed with water, air dried and crystallized from ethanol.

Ethyl 4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxylate (12a)

Yield 65%; m.p. 150–152°C; IR (KBr, cm^{-1}): 3396 (NH, br.), 1752, 1692 (2 C=O), 1613 (C=N), 1589 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.23 (t, 3H, CH_2-C<u>H</u>$_3$), 2.27 (s, 3H, CH_3), 3.83 (m, 5H, OCH_3 + <u>C</u>H$_2$-CH_3), 5.43 (s, 1H, CH of pyrimidine), 7.03–8.32 (m, 8H, Ar-H), 9.10 (s, 1H, CH of pyrazole), 9.17 (2s, 2H, 2 NH exchangeable with D_2O). MS (m/z %): 466 (0.1, M^+), 438 (8.4), 436 (27.8), 437 (25.9), 435 (78.3), 311 (4.8), 309 (13.6), 286 (34.4), 284 (100.0), 113 (12.8), 111 (33.7), 75 (17.3). Analysis: calc. for $C_{24}H_{23}ClN_4O_4$: C, 61.72; H, 4.97; N, 12.00%; found: C, 61.84; H, 4.98; N, 11.97%.

Ethyl 4-[1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate (12b)

Yield 68%; m.p. 182–185°C; IR (KBr, cm^{-1}): 3389, 3183 (2 NH), 1723 (C=O), 1672 (C=N), 1596 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 0.85 (t, 3H, CH_2-<u>C</u>H$_3$), 2.28 (s, 3H, CH_3), 3.81 (m, 5H, OCH_3 + <u>C</u>H$_2$-CH_3), 5.38 (s, 1H, CH of pyrimidine), 7.01–7.98 (m, 8H, Ar-H), 8.41 (s, 1H, CH of pyrazole), 9.63, 10.20 (2s, 2H, 2 NH exchangeable with D_2O). MS (m/z, %): 482 (2.5, M^+), 311 (42.8), 309 (100.0), 286 (12.4), 284 (30.7), 199 (10.6), 113 (16.3), 111 (46.7), 75 (27.3). Analysis: calc. for $C_{24}H_{23}ClN_4O_3S$: C, 59.68; H, 4.80; N, 11.60%; found: C, 59.50; H, 4.81; N, 11.55%.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4,7,8-hexahydro-2-oxo or thioxoquinazolin-5(6H)-one (13a,b). General method

A mixture of compound 2 (1.6 g, 0.005 mol), urea or thiourea (0.005 mol) and 1,3-cyclohexanedione (0.8 g, 0.007 mol) in absolute ethanol (20 mL) containing few drops of hydrochloric acid was refluxed for 3 h. After cooling, the obtained solid was filtered, air dried and crystallized from benzene.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4,7,8-hexahydro-2-oxoquinazolin-5(6H)-one (13a)

Yield 71%; m.p. 175–178°C; IR (KBr, cm^{-1}): 3340, 3200 (2 NH), 1710, 1692 (2 C=O), 1677 (C=N), 1594 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.13 (t, 2H, CH_2 of cyclohexanone), 1.89 (m, 2H, CH_2 of cyclohexanone), 2.49 (t, 2H, CH_2 of cyclohexanone), 3.80 (s, 3H, OCH_3), 5.42 (s, 1H, CH of pyrimidine), 6.50–7.91 (m, 8H, Ar-H), 8.90 (s, 1H, CH of pyrazole), 10.01 (s, 2H, 2 NH exchangeable with D_2O). Analysis: calc. for $C_{24}H_{21}ClN_4O_3$: C, 64.21; H, 4.72; N, 12.48%; found: C, 64.40; H, 4.71; N, 12.51%.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4,7,8-hexahydro-2-thioxoquinazolin-5(6H)-one (13b)

Yield 67%; m.p. 150–152°C; IR (KBr, cm^{-1}): 3365, 3158 (2 NH), 1707 (C=O), 1677 (C=N), 1588 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.04 (t, 2H, CH_2 of cyclohexanone), 1.25 (m, 2H, CH_2 of cyclohexanone), 2.48 (t, 2H, CH_2 of cyclohexanone), 3.82 (s, 3H, OCH_3), 5.49 (s, 1H, CH of pyrimidine), 6.58–7.58 (m, 8H, Ar-H), 8.90 (s, 1H, CH of pyrazole), 9.55, 10.97 (2s, 2H, 2 NH exchangeable with D_2O). Analysis: calc. for $C_{24}H_{21}ClN_4O_2S$: C, 62.00; H, 4.55; N, 12.05%; found: C, 61.81; H, 4.54; N, 12.08%.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-2-oxo or thioxoindeno [1,2-d] pyrimidin-5-one (14a,b). General method

A mixture of compound **2** (1.6 g, 0.005 mol), urea or thiourea (0.005 mol) and 1,3-indanedione (1

g, 0.007 mol) in absolute ethanol (20 mL) containing few drops of hydrochloric acid was refluxed for 3 h. After cooling, the solid obtained was filtered, air dried and crystallized from ethanol.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-2-oxo-indeno [1,2-d] pyrimidine-5-one (14a)

Yield 82%; m.p. 240-242°C; IR (KBr, cm^{-1}): 3369, 3107 (2 NH), 1722, 1680 (2 C=O), 1646 (C=N), 1584 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.49 (s, 1H, NH exchangeable with D_2O), 3.87 (s, 3H, OCH_3), 7.10–7.95 (m, 13H, Ar-H and CH of pyrimidine), 8.05 (s, 1H, CH of pyrazole), 9.95 (s, 1H, NH exchangeable with D_2O). Analysis: calc. for $C_{27}H_{19}ClN_4O_3$: C, 67.15; H, 3.97; N, 11.60%; found: C, 67.22; H, 3.98; N, 11.58%.

4-[1-(3-Chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl]-1,2,3,4-tetrahydro-2-thioxo-indeno [1,2-d] pyrimidine-5-one (14b)

Yield 79%; m.p. 230–232°C; IR (KBr, cm^{-1}): 3399, 3136, 3108 (2 NH), 1722 (C=O), 1679 (C=N), 1580 (C=C). Analysis: calc. for $C_{27}H_{19}ClN_4O_2S$: C, 64.99; H, 3.84; N, 11.23%; found: C, 64.73; H, 3.85; N, 11.27%.

Pharmacological activity

Chemicals and drugs

Carrageenan was purchased from Sigma Aldrich (St. Louis, MO, USA). Celecoxib was gifted from Sedico Pharmaceutical Co. (6 October city, Egypt). Selected compounds **2**, **3a,b**, **4–8**, **9a,b**, **10a,b**, **11a,b**, **12a,b**, **13a,b** and **14a,b** were evaluated as anti-inflammatory using human whole blood (HWB) assay and carragenan induced paw edema model.

Assessment of anti-inflammatory activity

HWB assay (19)

Heparinized blood samples were collected from the treated rats (n = 8), then, plasma were sep-

Table 1. Anti-inflammatory activities (*in vitro* and *in vivo*) of the tested compounds and reference standard at doses of 2.5 and 5 mg/kg after 4 h.

Compound	*In vitro* % Inhibition of plasma PGE		*In vivo* % Inhibition of RPE	
No.	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg
2	61.00	77.00	64.97	80.13
3a	68.98	93.12	76.98	94.85
3b	56.89	72.02	56.98	71.12
4	70.11	95.12	78.00	96.00
5	64.98	88.76	68.19	85.60
6	69.89	94.78	77.21	95.12
7	73.17	91.14	81.22	98.65
8	68.98	91.11	72.00	90.00
9a	63.34	78.98	67.98	84.16
9b	66.12	90.00	70.09	87.99
10a	59.16	75.18	59.96	76.12
10b	57.00	73.43	56.12	74.87
11a	72.00	97.00	79.13	97.65
11b	69.09	92.00	73.66	91.16
12a	71.98	96.98	78.99	97.00
12b	73.00	91.00	80.16	98.15
13a	65.87	89.46	69.70	86.11
13b	62.89	78.00	66.44	83.12
14a	69.75	92.98	74.95	92.90
14b	67.57	90.12	71.16	88.50
Celecoxib	92.88	97.55	96.44	99.93

Table 2. Ulcerogenic activity of tested compounds and reference standard at a dose of 5 mg/kg.

Compd. No.	Average no. of ulcers X ± S.E	Average no. of severity of ulcers Y ± SE	% of incidence of ulcer divided by 10	Ulcer index (UI)
2	1.16 ± 0.05	0.81 ± 0.011	2.80	2.1
3a	2.66 ± 0.24	2 ± 0.28	6	4.27
3b	2.16 ± 0.22	1.11 ± 0.12	5.11	4.12
4	1.44 ± 0.23	1.13 ± 0.11	4.32	3.22
5	1.12 ± 0.04	0.88 ± 0.01	3.33	2.21
6	2.40 ± 0.21	1.75 ± 0.21	5.82	4.11
7	1.12 ± 0.08	0.81 ± 0.021	3.11	2.31
8	1.08 ± 0.053	0.69 ± 0.01	2.82	2.3
9a	8.41 ± 0.16	4.19 ± 0.063	11	20
9b	1.19 ± 0.061	0.71 ± 0.021	2.90	2.11
10a	2.88 ± 0.39	2.11 ± 0.26	5.83	4.16
10b	2.56 ± 0.32	1.91 ± 0.23	5.81	4.12
11a	1.64 ± 0.11	1.13 ± 0.14	4.33	3.45
11b	1.22 ± 0.081	0.91 ± 0.029	3.17	2.36
12a	2.40 ± 0.41	1.64 ± 0.29	5.91	4.08
12b	1.12 ± 0.073	0.81 ± 0.029	3.01	2.21
13a	1.08 ± 0.054	0.69 ± 0.019	2.80	2.03
13b	1.66 ± 0.20	1.12 ± 0.12	4.35	3.05
14a	8.30 ± 0.11	4.18 ± 0.061	10	19
14b	1.10 ± 0.061	0.71 ± 0.021	2.90	2.11
Celecoxib	1.88 ± 0.20	1.33 ± 0.18	4.05	3

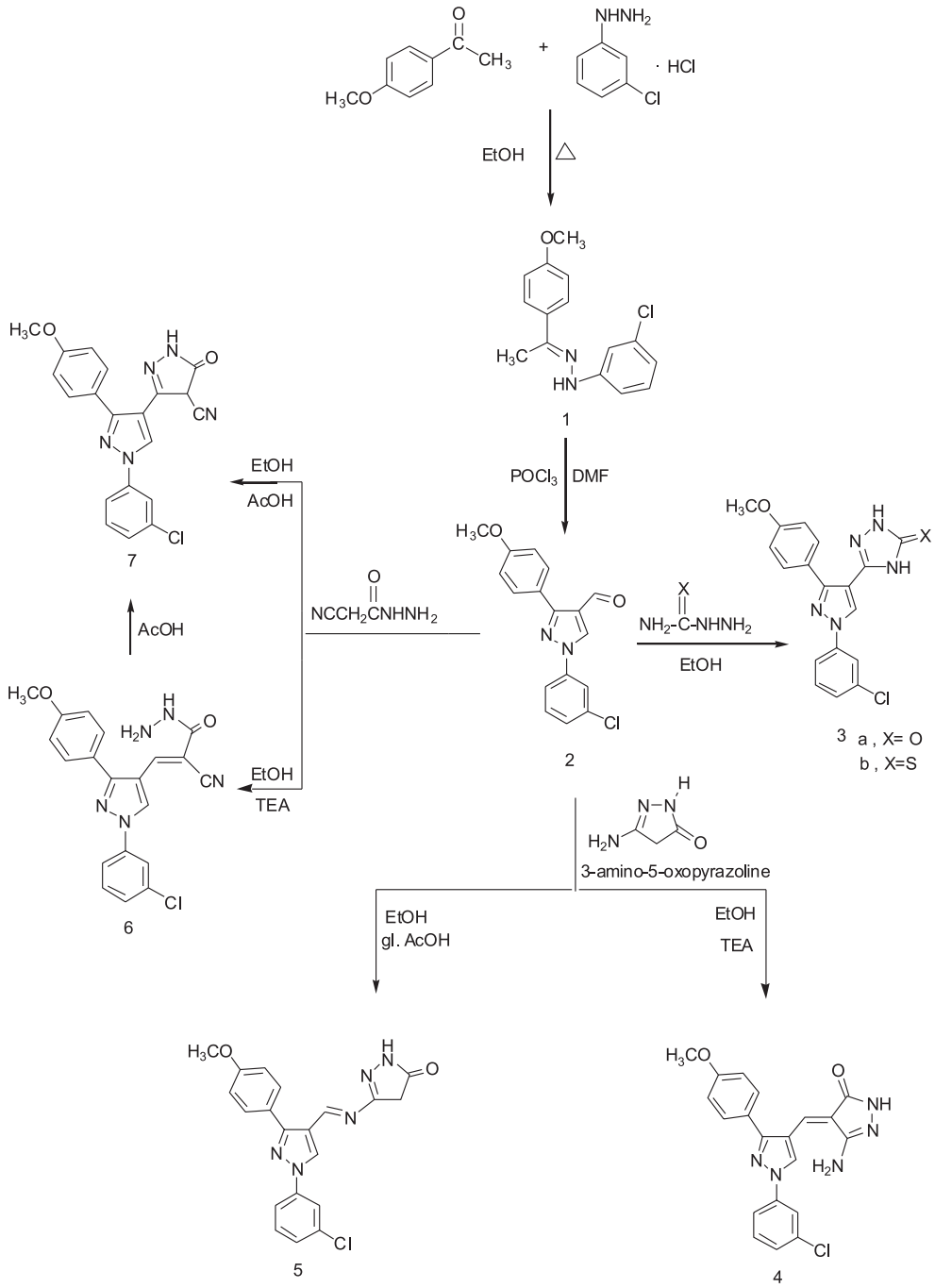
arated by centrifugation of these samples at 12000 × g for 2 min at 4°C and immediately stored frozen at –20°C until use, where PG was estimated by kits of immunoassay. The designs' correlate-EIA prostaglandin E_2 (PGE_2) kit is a competitive immunoassay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature, the excess reagents were washed away and the substrate was added. After a short incubation time, the enzyme reaction was stopped and the yellow color generated was read on a microplate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples (Table 1).

Carrageenan-induced rat paw edema (RPE) bioassay (*in vivo*)

The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of Winter et al. (20). Groups of adult male albino rats (150–180 g), each of 8 animals, were orally dosed with the test compounds at a dose level of 2.5 and 5 mg/kg one hour before carrageenan challenge. Foot paw edema was induced by sub-planter injection (done *sc* on the foot paw) of 0.05 mL of 1% suspension of carrageenan in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration, the animals were decapitated, blood was collected and the paws were rapidly excised. The average weight of edema was estimated for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Celecoxib (2.5 and 5 mg/kg) was employed as the standard reference (Table 1).

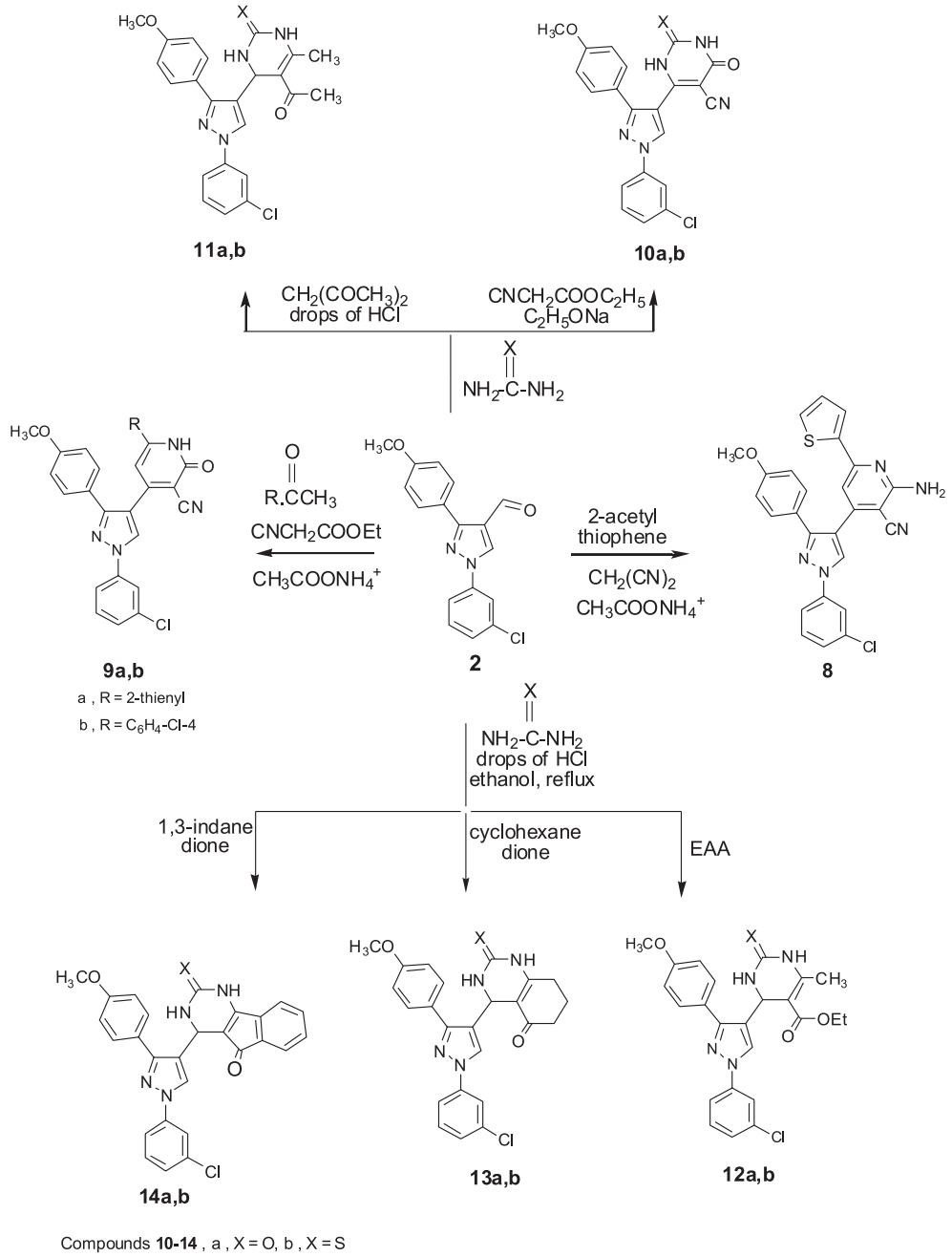
Ulcerogenic activity (23)

Groups of 10 male Wistar rats with weight between 150–175 g were used. They were starved 48 h prior to drug administration. The test compounds were administered orally in 10 mL/kg as aqueous suspension. Doses were used which are



Scheme 1.

highly active in the activity (5 mg/kg). The animals were sacrificed after 7 h. Stomachs were removed and placed on saline soaked filter paper until inspection. A longitudinal incision along the greater curvature was made with fine scissor. The stomach was inverted over the index finger and the presence or the absence of gastric irritation was determined. The presence of a single or multiple lesions (erosion, ulcer or perforation) was considered to be positive. The number of ulcers and the occurrence of hyperemia was noted to determine the ulcer index (Table 2).



Scheme 2.

RESULTS AND DISCUSSION

Synthesis of the desired 1-(3-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carboxaldehyde **2** started by condensation of p-methoxyacetophenone with m-chlorophenylhydrazine hydrochloride in refluxing ethanol, to afford hydrazone **1**. Vilsmeier-Haack reaction of **1** using phosphorus oxychloride in N,N-dimethylformamide afforded ultimately after hydrolysis, the desired key intermediate **2**, which was allowed to react with semicarbazide hydrochloride in the presence of sodium acetate to form **3a**, while the reaction with thiosemicarbazide in the presence of acetic acid gave **3b**. The key intermediate, **2** was reacted with 3-amino-5-oxo-2-pyrazoline in refluxing ethanol containing

few drops of TEA. The formyl function condensed with the active methylene in the ring forming the respective arylidene derivative **4**. However, when the reaction takes place in refluxing ethanol containing few drops of acetic acid, the formyl function condensed with the 3-amino group forming Schiff's base, **5**. Condensation of compound **2** with cyanoacetohydrazide in ethanol containing a few drops of TEA at room temperature gave compound **6**, which upon refluxing in ethanol containing a few drops of acetic acid was cyclized and gave the corresponding pyrazolinone derivative **7**. The same product **7** was isolated and identified when the reaction takes place in refluxing acetic acid between compound **2** and 2-cyanoacetohydrazide directly (Scheme 1). The spectral and microanalytical data for compounds **2–7** were consistent with their chemical structures. A new series of products containing pyridine derivatives **8** and **9a,b** were synthesized *via* the reaction of compound **2** with 2-acetylthiophene and/or p-chloroacetophenone with malononitrile or ethylcyanoacetate in the presence of ammonium acetate under reflux condition yielding the desired derivatives **8** and **9a,b**, respectively. On the other hand, another part of the research had focused on the synthesis of 2-oxo (thioxo) tetrahydropyrimidine derivatives. Cyclocondensation of aldehyde, **2** with urea or thiourea and ethylcyanoacetate in the presence of sodium ethoxide gave the corresponding 2-oxo (thioxo) pyrimidine derivatives **10a,b**. Furthermore, an acid-catalyzed three components condensation reaction in which a mixture of 1,3-dicarbonyl compounds (acetylacetone or ethylacetoacetate), the aldehydic compound **2** and urea or thiourea, in absolute ethanol and few drops of concentrated hydrochloric acid was refluxed to afford Biginelli compounds. It was a convenient route to achieve the preparation of the desired heterocyclic compounds **11a,b** and **12a,b**. Biginelli condensation was used once more to synthesize another target compounds. Thereby, reaction of 1,3-cyclohexanedione or 1,3-indanedione with urea or thiourea and compound **2** provided substituted hydroquinazoline or indeno [1,2-*d*] pyrimidine-2-oxo (thioxo) derivatives **13a,b** and **14a,b** (Scheme 2). Structures of target compounds, **8–14** were established on the basis of their elemental and spectral analyses.

Anti-inflammatory activity (*in vitro* and *in vivo*)

All the newly synthesized compounds and celecoxib, as a reference drug, were subjected to *in vitro* and *in vivo* anti-inflammatory studies using HWB assay (19) and carrageenan-induced RPE bioassay (20), respectively.

Most of the tested compounds at a dose level of 2.5 and 5 mg/kg showed significant anti-inflammatory activities with prostaglandin inhibition (Table 1).

Some general features could be concluded from the pharmacological screening as follows: It is favorable to have hybrids of pyrazole and pyrazolin-3-one directly attached (**7**, rat paw edema inhibition (RPEI) % = 81.2) without spacer groups as in **4** (CH=, RPEI % = 78.0) and 5 (CH=N, RPEI % = 68.2).

Compound **9b** was found to be more potent as anti-inflammatory than **9a**, respectively, which may be due to the presence of 4-chlorophenyl instead of 2-thienyl at C-6 of pyridine moiety.

Furthermore, pyrazoles **10a, 11a, 13a** and **14a** containing carbonyl group at C-2 of pyrimidine moiety were more potent anti-inflammatory than their counterparts, **10b, 11b, 13b** and **14b**, respectively; which have instead the more lipophilic, thioxo group.

Ulcerogenic activity

All compounds and celecoxib, as a reference drug, were studied for ulcerogenic activity [23]. The animals tolerated the tested compounds quite well and no mortalities have been recorded among them. The ulcer indices of all compounds are mainly in the safe level (UI = 2.10–4.27) except for compounds **9a** and **14a** which were highly ulcerogenic. (Table 2).

CONCLUSION

Various substituted pyrazole derivatives were prepared with the objective of developing better anti-inflammatory molecules. Most of the tested compounds at a dose level of 2.5 and 5 mg/kg showed significant anti-inflammatory activities with prostaglandin inhibition (*in vitro* and *in vivo*).

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