

Synthesis, anti-inflammatory, analgesic and antipyretic activities of novel pyrano[2,3-*c*]pyrazoles and related fused ring derivatives.

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Abstract. Objective; A series of some new pyrano[2,3-*c*]pyrazole and pyrano[2,3-*d*]pyrimidine derivatives were synthesized and evaluated for their anti-inflammatory, analgesic and antipyretic activities. The results showed that all compounds possessed promising anti-inflammatory activity. Compounds **6a**, **6b**, **8** and **9** have shown a potent anti-inflammatory activity more than Piroxicam reference drug. Whereas, compounds **4c**, **7b**, **7c** and **10** exhibited good analgesic activity compared to Piroxicam and compounds **4b** and **5c** showed excellent antipyretic activity more than Piroxicam. None of the tested compounds showed ulcerogenic effect.

[Afaf Kamal El-Din El-Ansary, Azza Taher Taher, Ahmed Abd El-Hamed El-Rahmany, Sally El Awdan. **Synthesis, anti-inflammatory, analgesic and antipyretic activities of novel pyrano[2,3-*c*]pyrazoles and related fused ring derivatives.** *J Am Sci* 2014;10(10):284-294]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 41

Key words: pyrano(2,3-*c*)pyrazole, pyrano(2,3-*d*)pyrimidine, anti-inflammatory, analgesic and antipyretic.

1. Introduction

Currently available non-steroidal anti-inflammatory drugs (NSAIDs) remain widely prescribed medications worldwide for the treatment of pain, fever, and swelling associated with arthritis [1, 2]. Most of their work by reducing the levels of prostaglandins by blocking cyclooxygenase enzyme [3-5], resulting in lower concentrations of prostaglandins as a consequence, inflammation and pain are reduced. However, long-term use of these drugs result in gastrointestinal (GI) side effects which are inseparable from their pharmacological activities such as ulceration, bleeding and renal toxicity [6-11]. Selective cyclooxygenases-2 (COX-2) inhibitors represent a new generation of anti-inflammatory drugs

as they demonstrated less gastrointestinal side effects than classical NSAIDs, which also inhibit the cytoprotective action of COX-1 in the gastrointestinal tract [12,13]. During the last decade, several selective COX-2 inhibitors (coxibs) have reached the market such as piroxicam (Feldene®) and meloxicam (Mobic®) [14]. Despite of the relatively safe pharmacological profile of selective COX-2 inhibitors, these drugs showed a risk of adverse cardiovascular events such as myocardial infarction[15]. Consequently, development of novel compounds having anti-inflammatory, analgesic and antipyretic activities with an improved safety profile is a great deal of interest to many researchers.

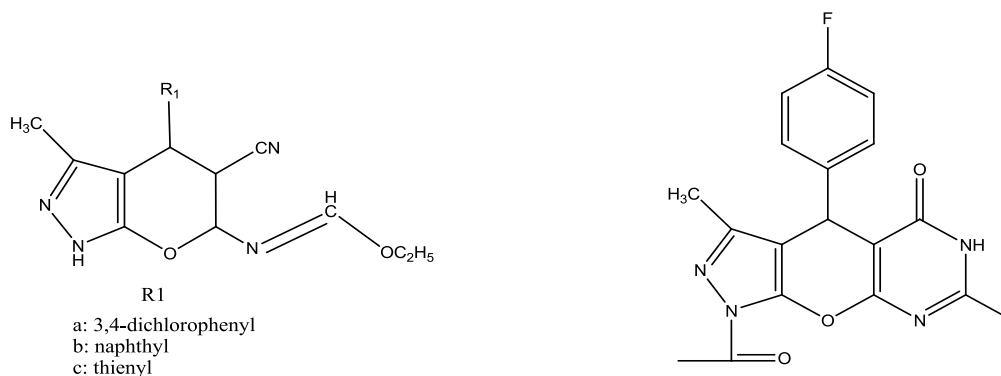
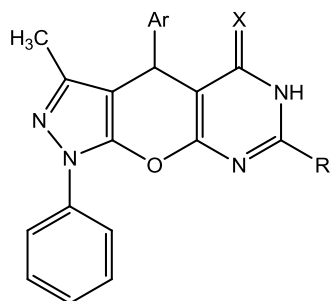


Fig.1. Representative examples of some pyranopyrazole and pyrazolopyranopyrimidine derivatives exhibit anti-inflammatory activity.

Literature survey revealed that, pyranopyrazole and fused pyrimidines especially pyrazolopyranopyrimidine derivatives are known to exhibit unique potential anti-inflammatory, analgesic and antipyretic activities Fig.1 [16,17]. So, they gained much attention as important pharmacophore



and privileged structure in medicinal chemistry. In the current work, the challenge was to design and synthesize novel derivatives of pyranopyrazole and pyrazolopyranopyrimidine to maintain sufficient efficacies in human pain models and to reduce side effects. Fig. 2.

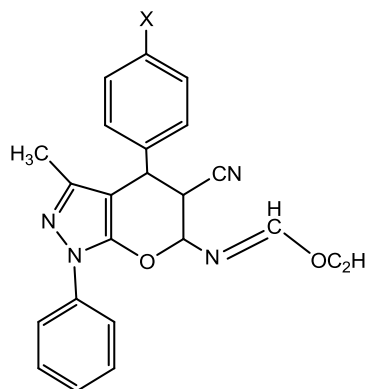


Fig. 2. The designed target compounds.

2. Materials and methods

2.1. Chemistry

Melting points were determined on Stuart apparatus and the values given are uncorrected. IR spectra were recorded on Shimadzu IR 435 spectrophotometer and values were represented in cm^{-1} . $^1\text{H-NMR}$ and $^{13}\text{C NMR}$ were carried out on Varian Gemini 300 MHz spectrophotometer at The Microanalytical center, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. The electron impact (EI) mass spectra were recorded on Hewlett Packard 5988 spectrometer at The Microanalytical center, Cairo University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates eluting solvents are chloroform: methanol 9.5:0.5 and benzene: acetone 8:2, containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. Elemental microanalyses were performed on a VARIO ELEMENTAR at the center for Biotechnology, AL- Azhar University, Cairo, Egypt, and were within $\pm 0.4\%$.

Nofal *et al.*, [17] reported preparation of 6-Amino-4-aryl-3-methyl-1-phenyl-1,4-dihydro pyrano[2,3-*c*]pyrazole-5-carbonitrile (**3_{a-e}**).

2.1.1. Synthesis of 4-aryl-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6] pyrano[2,3-*d*]pyrimidine-5(1H)-one (**4_{a-c}**)

General procedure

A mixture of compound **3_{b,c,e}** (0.01mol) and formic acid (32mL) was refluxed for 10 hours. Excess formic acid was then removed under reduced pressure,

the residue washed twice with water (20mL), dried and crystallized from absolute ethanol.

2.1.1.1. 4-(4-Fluorophenyl)-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano [2,3-*d*]pyrimidine-5(1H)-one (**4_a**)

Yellow powder; yield 27%; mp: 133-135 °C; IR (KBr, cm^{-1}): 1690 (C=O), 2922 (CH aliphatic), 3412(NH); $^1\text{H-NMR}$ (DMSO-*d*₆): δ 2.05 (s, 3H, CH₃), 4.93 (s, 1H, pyrano), 6.89-7.22 (m, 5H, ArH), 7.32(s, 1H, H-pyrimidine), 7.25, 7.71 (2d, 4H, *J*=8.4Hz), 12.10 (1s, 1H, 1NH Exchangeable by D₂O); Anal. Calcd for C₂₁H₁₅FN₄O₂ (374.36): C, 67.37; H, 4.04.; N, 14.97 Found C, 67.56; H, 4.17; N, 15.24.

2.1.1.2. 4-(4-Chlorophenyl)-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6] pyrano[2,3-*d*]pyrimidine-5(1H)-one (**4_b**)

Pale yellow powder; yield 51%; mp: 95-97 °C; IR (KBr, cm^{-1}): 1663 (C=O), 2924 (CH aliphatic), 3419 (NH); $^1\text{H-NMR}$ (DMSO-*d*₆): δ 2.05 (s, 3H, CH₃), 4.20 (s, 1H, pyrano), 7.25-7.55 (m, 5H, ArH), 7.34 (s, 1H, H-pyrimidine), 7.14, 7.72 (2d, 4H, *J*=7.8 Hz, ArH), 7.95 (s, 1H, 1NH Exchangeable by D₂O); MS (EI) *m/z*: 390.00 (M⁺, 8.96%), 391.00 (M+H, 28.36%), 392.00 (M+2, 20.52%), 137.15 (100 %); Anal. Calcd for C₂₁H₁₅ClN₄O₂ (390.82.): C, 64.54; H, 3.87; N, 14.34. Found. C, 64.63; H, 3.90; N, 14.72.

2.1.1.3. 4-(4-Bromophenyl)-3-methyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6] pyrano [2,3-*d*]pyrimidine-5(1H)-one (**4_c**)

Orange powder; yield 41%; mp: 99-101 °C; IR (KBr, cm^{-1}): 1666 (C=O), 2922 (CH aliphatic), 3419 (NH); $^1\text{H-NMR}$ (DMSO-*d*₆): δ 2.29 (s, 3H, CH₃), 4.19 (s, 1H, pyrano), 7.25-7.48 (m, 5H, ArH),

7.42, 7.70 (2d, 4H, $J=7.5$ Hz, ArH), 8.50 (s, 1H, H-pyrimidine), 9.98 (s, 1H, 1NH Exchangeable by D₂O); MS (EI) m/z : 434 (M⁺, 30.53%), 435.10 (M+H, 35.34%), 436.10 (M+2, 31.23%), 210.90 (100%); Anal. Calcd for C₂₁H₁₅BrN₄O₂ (435.27): C, 57.95; H, 3.47; N, 12.87. Found: C, 58.19; H, 3.58; N, 13.13.

2.1.2. Synthesis of 4-aryl-3-methyl-1-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano [2,3-d]pyrimidine-5-amine (5_{a-c})

General procedure

To a mixture of compound 3_{b,c,e}, formamide (10mL), formic acid (5mL) and dimethylformamide (5mL), was heated under reflux for 10 hours. The reaction mixture was then cooled, the formed precipitate filtered, washed twice with water, (20mL) dried and crystallized from absolute ethanol.

2.1.2.1. 4-(4-Fluorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano [2,3-d]pyrimidine-5-amine (5_a)

Brown powder; yield 40%; mp: 88-90 °C; IR (KBr, cm⁻¹): 2922 (CH aliphatic), 3412 (NH₂); ¹H-NMR (DMSO-*d*₆): δ 1.95 (s, 3H, CH₃), 4.20 (s, 1H, pyrano), 7.08-7.72 (m, 5H, ArH) 7.38, 7.98 (2d, 4H, $J=8.7$ Hz, ArH), 7.41(s, 1H, CH of pyrimidine), 7.44 (s, 2H, NH₂ Exchangeable by D₂O); Anal. Calc for C₂₁H₁₆FN₅O (373.38): C, 67.19; H, 4.83; N, 18.66. Found: C, 67.38; H, 4.09; N, 18.97.

2.1.2.2. 4-(4-Chlorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano [2,3-d]pyrimidine-5-amine (5_b)

Dark brown powder; yield 51%; mp: 125-127 °C; IR (KBr, cm⁻¹): 2924 (CH aliphatic), 3414 (NH₂); ¹H-NMR (DMSO-*d*₆): δ 1.91 (s, 3H, CH₃), 4.96 (s, 1H, pyrano), 7.09-8.61 (m, 5H, ArH), 7.35, 7.71 (2d, 4H, $J=8.7$ Hz, ArH), 7.68 (s, 2H, NH₂, Exchangeable by D₂O), 7.70 (s, 1H, CH of pyrimidine); MS (EI) m/z : 389.15 (M⁺, 0.57%), 390.20 (M+H, 0.28%), 391.50 (M+2, 0.36%), 77.05 (100%); Anal. Calc for C₂₁H₁₆ClN₅O (389.84): C, 64.37; H, 4.63; N, 17.87. Found C, 64.43; H, 4.68; N, 18.12.

2.1.2.3. 4-(4-Bromophenyl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5-amine (5_c)

Yellowish brown powder; yield 46%; mp: 108-110 °C; IR (KBr, cm⁻¹): 2924 (CH aliphatic), 3419 (NH₂); ¹H-NMR (DMSO-*d*₆): δ 2.35 (s, 3H, CH₃), 4.73 (s, 1H, pyrano), 5.98 (s, 2H, NH₂, Exchangeable by D₂O), 7.23-8.00 (m, 5H, ArH), 7.40, 7.46 (d, 2H, $J=6.6$ Hz, ArH), 7.49, 7.69 (d, 2H, $J=8.1$ Hz, ArH), 8.61(s, 1H, CH of pyrimidine); MS (EI) m/z : 435.90 (M+3, 44.79%), 436.90 (M+4, 40.63%), 77.00 (100%); Anal. Calc for C₂₁H₁₆BrN₅O (434.29): C, 57.81; H, 4.16; N, 16.05. Found C, 57.92; H, 4.22; N, 16.38; ¹³C NMR (DMSO): 13.15, 26.75, 79.88, 100.75, 119.30, 120.33, 123.53, 124.16 (2C),

128.75(2C), 129.05(2C), 130.14(2C), 131.29, 132.93, 139.15, 140.90 146.90, 164.68.

2.1.3. Synthesis of 4-aryl-3,7-dimethyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-one (6_{a,b})

General procedure

Method A:

A mixture of 3_{d,e} (0.01mol) in acetic acid (20mL) and acetic anhydride (15mL) was heated under reflux for 15 hours. The reaction mixture was then cooled and poured into ice-water (20mL). The formed precipitate was filtered, dried and crystallized from absolute ethanol.

Method B:

A mixture of either 3_{d,e} (0.01mol), acetic anhydride (20mL) and H₂SO₄ (1mL) was heated under reflux for one hour and left overnight. The reaction mixture was poured into ice-cold water (20mL), the formed precipitate filtered, dried and crystallized from absolute ethanol.

2.1.3.1. 4-(2-Bromophenyl)-3,7-dimethyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-One (6_a)

White micro crystals; yield method A: 40%, method B: 29%; mp: >300 °C; IR (KBr, cm⁻¹): 1670 (C=O of amide), 2920 (CH aliphatic), 3440 (NH); ¹H-NMR (DMSO-*d*₆): δ 1.90 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 5.42 (s, 1H, pyrano), 7.09-7.33 (m, 5H, ArH), 7.52 (d, 1H, ArH), 7.54(m, 2H, ArH), 7.75 (d, 1H, ArH), 12.58 (s, 1H, NH Exchangeable by D₂O); Anal. Calcd for C₂₂H₁₇BrN₄O₂ (449.30): C, 58.81; H, 3.81; N, 12.47. Found C, 59.02; H, 3.88; N, 12.75.

2.1.3.2. 4-(4-Bromophenyl)-3,7-dimethyl-1-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-One (6_b)

White crystals; yield method A: 45%, method B: 22%; mp: >300 °C; IR (KBr, cm⁻¹): 1689 (C=O of amide), 2924 (CH aliphatic), 3437 (NH); ¹H-NMR (DMSO-*d*₆): δ 1.90 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 5.04 (s, 1H, pyrano), 7.31-7.72 (m, 5H, ArH), 7.24, 7.75 (2d, 4H, $J=7.8$ Hz, ArH), 12.64 (1s, 1H, NH Exchangeable by D₂O); MS (EI) m/z : 448.15 (M⁺, 14.66%), 449.15 (M+H, 4.19%), 450.15 (M+2, 12.19%), 293.20 (100%); Anal. Calcd for C₂₂H₁₇BrN₄O₂ (449.30): C, 58.81; H, 3.81; N, 12.47. Found C, 59.15; H, 3.93; N, 12.56; ¹³C NMR (DMSO): 13.01, 17.00, 40.36, 115.62, 117.86, 118.31, 124.56(2C), 126.17(2C), 128.74(2C), 128.90(2C), 129.70, 136.73, 138.05, 144.00, 151.69, 161.40, 162.94, 166.31.

2.1.4. Synthesis of 6-[[aryl methylene]amino]-3-methyl-1-phenyl-4-(4-substituted phenyl)-1,4-dihydropyrazolo[2,3-*c*]pyrazole-5-carbonitrile (7_{a-c})

General procedure

A mixture of compound 3_{a,e} (0.01mol) and the appropriate aldehyde (0.01mol) was fused for 3-5

hours. The reaction mixture was then cooled and washed twice with diethyl ether (20mL). The solid mass was filtered and crystallized from methanol.

2.1.4.1. 6-[[Furan-2-ylmethylene]amino]-3-methyl-1,4-diphenyl-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (7_a)

Dark brown needle crystals; yield 86%; mp: 175-177°C; IR (KBr, cm⁻¹): 2222(CN), 2924 (CH aliphatic); ¹H-NMR (DMSO-*d*₆): δ 2.10 (s, 3H, CH₃), 5.01 (s, 1H, pyrano), 6.41(d, 2H, ArH), 6.92 (d, 1H, ArH), 7.26-7.89 (m, 10H, ArH), 8.28 (s, 1H, N=CH); MS (EI) m/z: 406.35 (M⁺, 1.15%), 407.45 (M+H, 0.74%), 91.10 (100%); Anal. Calcd for C₂₅H₁₈N₄O₂ (406.44): C, 73.88; H, 4.46; N, 13.78. Found: C, 74.07; H, 4.59; N, 14.11.

2.1.4.2. 6-[[4-Bromobenzylidene]amino]-3-methyl-1,4-diphenyl-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (7_b)

Brownish red crystals; yield 81%; mp: 125-127°C; IR (KBr, cm⁻¹): 2212 (CN), 2920(CH aliphatic); ¹H-NMR (DMSO-*d*₆): δ 2.29 (s, 3H, CH₃), 3.70 (s, 1H, pyrano), 7.00-8.01(m, 14H, ArH), 8.61 (s, 1H, N=CH); Anal. Calcd for C₂₇H₁₉BrN₄O (495.37): C, 65.46; H, 3.87; N, 11.31. Found: C, 65.61; H, 3.98; N, 11.49.

2.1.4.3. 4-(4-Bromophenyl)-6-[[4-chlorobenzylidene]amino]-3-methyl-1-phenyl-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (7_c)

Dark red crystals; yield 85%; mp: 138-140°C; IR (KBr, cm⁻¹): 2225(CN), 2900(CH aliphatic); ¹H-NMR (DMSO-*d*₆): δ 2.06 (s, 3H, CH₃), 4.85 (s, 1H, pyrano), 7.01-7.80 (m, 14H, N=CH & ArH); MS (EI) m/z: 528.25 (M⁺, 0.55%), 530.15 (M+2, 0.60%), 77.10 (100%); Anal. Calcd for C₂₇H₁₈BrClN₄O (529.82): C, 61.21; H, 3.42; N, 10.57. Found: C, 61.29; H, 3.51; N, 10.88.

2.1.5. Synthesis of ethyl N-5-cyano-4-(4-fluorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-c]pyrazol-6-ylformimidate (8)

General procedure

A mixture of compound 3_b (3.46g, 0.01mol), triethylorthoformate (1.48g, 1.66mL, 0.01mol) and acetic anhydride (16mL) was heated under reflux for 2 hours. The solvent was then removed under reduced pressure and the residue crystallized from absolute ethanol.

Yellowish white powder; yield; 75%; mp: 160-162 °C; IR (KBr, cm⁻¹): 2212 (CN), 2900 (CH aliphatic); ¹H-NMR (DMSO-*d*₆): δ 1.23 (t, 3H, CH₃), 1.79 (s, 3H, CH₃), 4.34 (q, 2H, CH₂), 4.99 (s, 1H, pyrano), 7.19-7.43 (m, 5H, ArH), 7.36 (d, 2H, *J*=8.4 Hz), 7.49 (d, 2H, *J*=8.4 Hz), 8.53 (s, 1H, CH-OC₂H₅); MS (EI) m/z: 401.15 (M-1, 6.36%), 402.15 (M⁺, 37.91%), 403.10 (M+H, 10.97%), 404.05 (M+2, 1.70%), 405.05 (M+3, 0.18%), 307.10 (100%); Anal.

Calcd for C₂₃H₁₉FN₄O₂ (402.42): C, 68.65; H, 4.76; N, 13.92. Found: C, 68.77; H, 4.82; N, 13.89.

2.1.6. Synthesis of 6-amino-4-(4-fluorophenyl)-5-imino-3-methyl-1-phenyl-1,4, 5,6-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine (9)

General procedure

A mixture of compound 8 (4g, 0.01mol), absolute ethanol (25mL) and 99% hydrazine hydrate (5mL) was stirred for one hour at room temperature and left overnight. The formed precipitate was filtered, dried and crystallized from methanol.

Yellow powder; yield 23%; mp: 110-112 °C; IR (KBr cm⁻¹): 2935 (CH aliphatic) 3147 (CH aromatic), 3334, 3385 (NH &NH₂); ¹H-NMR (DMSO-*d*₆): δ 2.18 (s, 3H, CH₃), 3.43 (s, 1H, pyran), 6.72-7.71 (m, 5H, ArH), 7.04 (d, 2H, *J*=7.8Hz), 7.21, 7.31 (d, 2H, *J*=7.8Hz), 7.38 (s, 2H, NH₂, exchangeable by D₂O), 7.94 (s, 1H, pyrimidine), 10.32 (s, 1H, NH, exchangeable by D₂O); MS (EI) m/z: 386.50 (M-2, 0.16%), 80.00 (100%); Anal. Calcd for C₂₁H₁₇FN₆O (388.39): C, 64.94; H, 4.41; N, 21.64. Found: C, 65.22; H, 4.53; N, 21.88.

2.1.7. Synthesis of 11-(4-fluorophenyl)-10-methyl-8-phenyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-*e*] [1,2,4]triazolo[1,5-*c*]pyrimidine (10)

General procedure

A mixture of compound 9 (4.04g, 0.01mol) and formic acid (32mL) was heated under reflux for 10 hours then cooled. The reaction mixture was poured into ice-cold water (10mL). The formed precipitate was filtered, washed several times with water, dried and crystallized from methanol.

Reddish yellow powder; yield 23 %, mp: 195-197° C; IR (KBr, cm⁻¹): 1600 (C=N), 2924 (CH aliphatic), 3074 (CH aromatic); ¹H-NMR (DMSO-*d*₆): δ 2.06 (s, 3H, CH₃), 3.95 (s, 1H, pyrano), 7.24-8.03 (m, 9H, ArH), 7.99 (s, 1H, triazole), 8.64 (s, 1H, pyrimidine); MS (EI) m/z: 398.30 (M⁺, 73.57%), 342.30 (100%); Anal. Calcd for C₂₂H₁₅FN₆O (398.39): C, 66.33; H, 3.80; N, 21.09. Found: C, 66.42; H, 3.76; N, 21.32.

2.2. Pharmacology

Pharmacological screening of novel synthesized compounds was carried-out at the National Research Centre, Pharmacology Department, Egypt. Anti-inflammatory activities of all novel synthesized compounds were assessed by utilizing carrageenan-induced rat's paw edema model[18-20], while analgesic activities were investigated by Tail Flick test[21]. Additionally, antipyretic activities were achieved by The subcutaneous injection of Brewer's yeast suspension [22,23] and acute ulcerogenicity was evaluated relative to piroxicam drug as positive control[25].

2.2.1. Acute Anti-inflammatory procedure (Carrageenan-induced paw edema):

The carrageenan-induced rat paw edema is one of the most commonly employed techniques for screening and evaluation of anti-inflammatory drugs. It is based upon the ability of the drugs to inhibit the edema induced in the hind paw of rats, by injecting a small amount of the edemogen (a polysaccharide: carrageenan) into the plantar tissue of the paw following to Winter method[18].

Procedure

Adult male albino rats of 120–150 g body weight were obtained from the animal house of National Research Centre, Cairo Egypt. Animals were kept in separate cages, six animals each, under hygienic conditions in temperature-controlled rooms at 20°C. Animals were allowed free access to standard laboratory diet and water, and they were maintained at a 12 h light/dark cycle. Work was done according to internationally accepted principles for laboratory animal's use and care in European Community Guidelines and Ethical Committee of National Research Centre

Approval was obtained.

Drugs

Piroxicam (Pfizer Company, Egypt), Carrageenan (Sigma, USA),

Anti-inflammatory assay

The anti-inflammatory activity of the newly synthesized compounds from **4a-c** to **10** were investigated in comparison with piroxicam according to paw edema method. The animals were randomly divided into groups of six animals each and were fasted for 18 h before the experiment, with free access to water. Standard drug piroxicam was administered orally at 20 mg/kg. Carrageenan solution in saline (1%, 0.1 mL) was injected into sub-planter region of right hind paw of each rat; the left hind paw volume was injected with 0.1 mL saline; 1 h after oral administration of the test compounds at dose level of 20 mg kg⁻¹ [26], (the doses were calculated according to the molecular weight) the left hind paw volume was measured using Plethysmometer. The percent edema inhibition was calculated from the mean difference between the two paws using a Plethysmometer (7141: UGO Basile, Comerio, Italy)[27] Each value represents the mean ± SEM relative to the standard. The mean increase of paw volume at each time interval was compared with that of control group.

The percentage inhibition values were calculated according to the formula:

% Anti-inflammatory activity = $(1 - Rt/Rc) \times 100$ (Rt = result of tested group; Rc = result of tested control).

2.2.2. Analgesic activity evaluation

Procedure

Analgesic responses were determined using the tail-flick test[21]. To measure the latency of the tail-flick response, Albino rats allocated to different groups consisting of animals. Each animal was placed gently held with the tail put on the apparatus (Ugo Basile, USA) for radiant heat stimuli. The tail flick response was elicited by applying radiant heat to the dorsal surface of the mouse-tail. Each animal was placed gently on the tail flick such that the tail is subjected to the infra red beam. Latency to exhibit nociceptive responses, such as removing the tail was determined at 30, 60, 90 min after administration of test substances or saline. Saline was administered in one group of animals subcutaneously (s.c.) and served as control. The time was measured in minutes from initial heat source activation until tail withdrawal was recorded. The mean of two measures was used for each experimental animal as the tail withdrawal latency. All drugs were injected orally 30 minutes before placing the animal on the hot plate at dose level of 20 mg kg⁻¹. The data represents the mean ± standard error of the mean (n = 6). Values represent the mean ± S.E. of six animals for each groups.

2.2.3. Antipyretic activity

Principle:

The subcutaneous injection of Brewer's yeast suspension is known to produce fever in rats. A decrease in the elevated body temperature can be achieved by administration of compounds with antipyretic activity [22]

Procedure

One ml/100 g body weight of 44% yeast suspension was administered by an intramuscular injection into each animal of all the tested groups. The site of injection was then massaged to spread the suspension into the tissues. Before yeast injection rectal temperature was recorded for all groups. The rectal temperature measured 18 hours following the yeast injection serves as the basic line of elevated body temperature, to which the anti-pyretic effect will be compared. At that specific time (18 hours after yeast injection) drugs were administered [22]. Rectal temperature was recorded by a multichannel electric thermometer (TMP 812 Digital Thermometer, Ugo Basile, Comerio, Italy) 1 and 2 h after administration of drugs [23]. The increase in rectal temperature at different times in respect to the values before administration of the yeast was calculated.

2.2.4. Ulcerogenic effect in rats

Procedure

Acute ulcerogenicity was determined according to the method of Szelenyi [24]. Rats were fasted for eighteen hours before the experiment. Drugs were orally given in all groups (25.5-37 mg/kg body weight). Five hours later, rats were sacrificed, and stomachs were removed opened along the greater

curvature and the number of ulcers assessed. To determine the number and severity of the gastric lesions, immediately after sacrifice the stomachs of the animals were opened and rinsed with 5 ml saline. The stomachs were carefully examined under a stereoscopic microscope (Metrimpex-PZ Labimex, Budapest, Hungary)[24,25]. The number of lesions was determined and the mean \pm SEM for each experimental group was presented. The technician who performed the scoring procedure did not know the treatment to which the animals had been submitted.

3. Results and Discussion

3.1. Chemistry

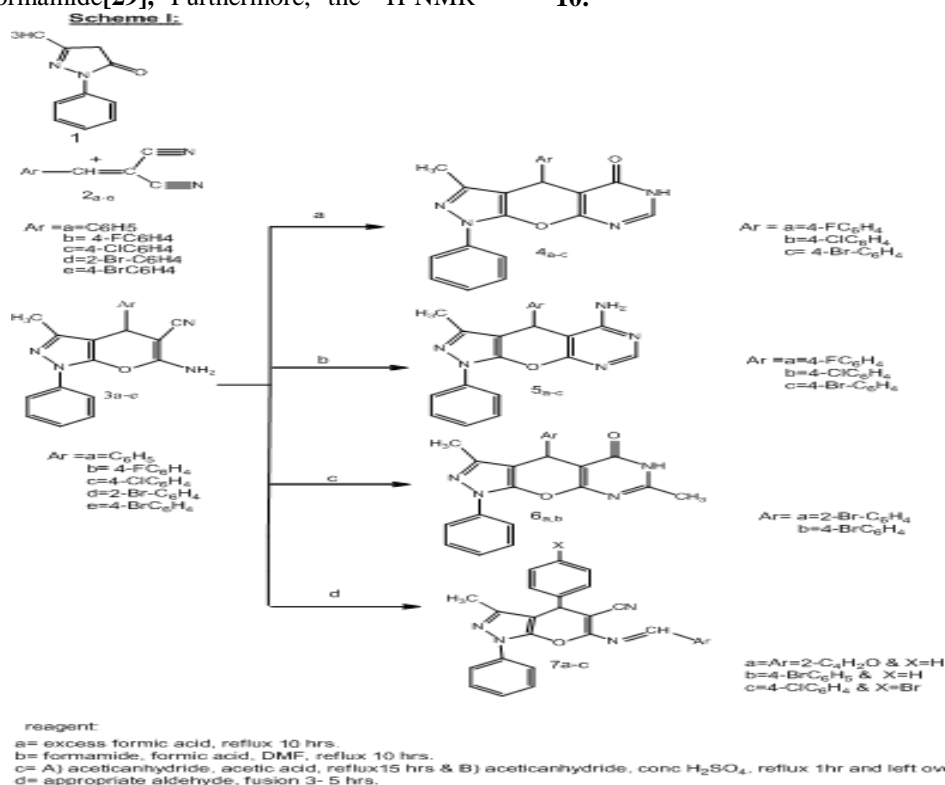
The reaction sequence employed for the preparation of target compounds from **4_{a-c}** to **10** was shown in Charts 1&2. Starting compounds **3_{a-e}** were prepared in 82- 96% yields by reaction of 3-methyl-1-phenyl-5-pyrazolone (**1**) commercially available and appropriate arylidinemalononitrile (**2**) as reported procedure[17,28], compounds **4_{a-c}** were synthesized through the reaction of **3_{b,c,e}** with excess formic acid[29,30]. The IR spectra of compounds **4_{a-c}** showed disappearance of absorption bands for (NH₂) and appearance of additional absorption bands in the range of 1663-1690 cm⁻¹ which confirmed the presence of carbonyl group (C=O), compound **5_{a-c}** were obtained by heating of **3_{b,c,e}** with formamide, formic acid and dimethylformamide[29], Furthermore, the ¹H-NMR

spectra of **5_{a-c}** showed a singlet signal for pyrimidine ring in the range of 7.41-8.61 ppm, respectively. On the other hand, synthesis of target compounds **6_{a,b}** were obtained successfully by acylation of compounds **3_{a,e}** with acetic anhydride in acetic acid/ or sulfuric acid[31] and compounds **7_{a-c}** were prepared fusion of compounds **3_{a,e}** with aromatic aldehyde. The structures of all novel synthesized compounds were determined by spectral and microanalytical analyses. The ¹H-NMR spectra of compounds **4_{a-c}** - **7_{a-d}** have shown an additional peaks around δ 6.89-8.61 ppm corresponding to the aromatic protons.

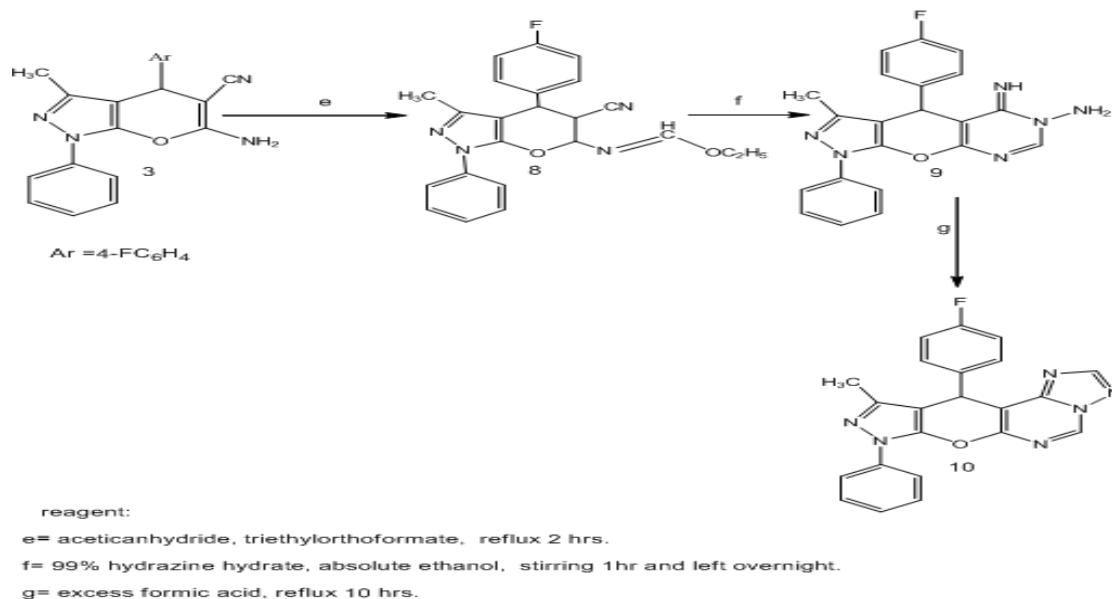
Moreover, in Chart 2 compound **8** was prepared by reaction of **3_b** with triethylorthoformate[31], compound **9** was synthesized by reaction **8** with 99% hydrazine hydrate in the presence of absolute ethanol[31,32] and compound **10** was obtained by reaction of **9** with excess formic acid[32,33]. The IR spectra exhibited the expected bands for the characteristic groups as CH₃ stretching and NH &NH₂ at 3334, 3385 cm⁻¹ which are present in the compounds **10**. The ¹H-NMR of the aromatic and aliphatic protons were observed in the expected regions and mass spectra data showed the molecular ion peak of the target final compounds.

Scheme 1. Synthesis of the target compounds from **4_{a-c}** to **7_{a-c}**.

Scheme 2. Synthesis of the target compounds **8, 9** and **10**.



Scheme II:



3.2. Pharmacological evaluation

3.2.1. Results of in-vivo anti-inflammatory screening:

All the newly compounds were subjected for their anti-inflammatory activities using carrageenan-induced rat's paw edema method. In general, all compounds exhibited promising anti-inflammatory activity by oral administration at a dose level of 20 mg kg⁻¹ compared to reference drug piroxicam (20 mg kg⁻¹). The relative percentage inhibition of edema recorded values around 83.50% (compound 9) to 42.54% (compound 4c), while the reference standard showed 62.17% inhibition of edema after four hours.

Compound 9 possessed an excellent anti-inflammatory activity with potency value 83.50% after four hours. This may be attributed to the substituted phenyl ring at 4-position gave rise to an increased anti-inflammatory activity[34] and Introducing of a pyrimidine ring fused to the pyranopyrazole moiety increases the anti-inflammatory activity[16]. Additionally, compounds 6a, 6b and 8 exhibited most prominent and consistent anti-inflammatory activity with rapid onset of action and sustained duration till four hours. The data was represented in table 1,2.

Table 1: Anti-inflammatory evaluation of tested compound.

Group	% Change from baseline			
	1h	2h	3h	4h
Control	71.69±9.941	105.2±13.98	119.3±12.236	125.1±11.12
Piroxicam	27.89±8.113*	33.86±2.365*	45.83±6.618*	47.32±6.750*
4a	50.82±7.922	70.40±5.568	72.72±8.523*	68.75±4.235*
4b	47.32±6.190	68.19±7.667	47.48±2.365*	60.02±7.539*
4c	111.4±2.081 [⊙]	94.72±4.019	91.91±10.25	71.87±3.172*
5a	53.02±7.374	77.01±8.236	45.88±4.025*	49.49±5.322*
5b	41.65±2.758	63.91±4.544	51.69±6.965*	47.57±2.439*
5c	59.69±6.356	89.62±10.325	65.70±2.289*	57.67±6.537*
6a	57.65±5.458	79.10±6.598	42.97±5.236*	32.73±1.262*
6b	87.43±8.657 [⊙]	97.05±10.235	70.71±12.235*	46.28±2.356*
7a	21.64±2.321*	42.92±5.064*	50.66±4.929*	53.98±6.420*
7b	37.53±3.895	51.61±4.365*	60.08±8.964*	71.29±8.792*
7c	22.50±3.214*	28.35±3.256*	35.42±8.057*	53.51±3.652*
8	57.42±3.872	60.29±5.776	49.81±4.469*	40.72±4.743*
9	34.04±5.088	49.41±6.620*	44.99±3.985*	20.63±1.468*
10	46.43±5.767	55.73±5.475*	52.16±3.844*	52.30±4.250*

The data represents the mean ± standard error of the mean (n = 6). Values represent the mean ± S.E. of six animals for each groups. * P< 0.05: Statistically significant from Control. (One way Anova followed by Tukey test).

Table 2: % Inhibition of edema of tested compounds after 1,2,3 and 4 hrs.

Group	% Inhibition (Potency)			
	1h	2h	3h	4h
Piroxicam	61.09	67.81	61.58	62.17
4a	29.11	33.07	39.04	45.04
4b	33.99	35.18	60.20	52.02
4c	-	9.96	22.95	42.54
5a	26.04	26.79	61.54	60.43
5b	41.90	39.24	56.67	61.97
5c	16.73	14.80	44.92	53.90
6a	19.58	24.80	63.98	73.83
6b	-	7.747	40.72	63.00
7a	69.81	59.20	57.53	56.85
7b	47.64	50.94	49.63	43.01
7c	68.61	73.05	70.31	57.22
8	19.90	42.69	58.24	67.45
9	52.51	53.03	48.43	83.50
10	35.23	47.02	48.43	58.19

3.2.2. Results of analgesic screening:

Potential analgesic activity of the novel compounds was evaluated following Tail Flick method at a dose of 20 mg kg⁻¹. In this method the analgesic responses were determined by measuring the time from initial heat radiation till latency of the tail-flick response. The tail flick response was elicited by applying radiant heat to the dorsal surface of the mouse-tail. As shown in table 3,4. Compounds **4c**, **7b**, **7c** and **10** showed a significant analgesic activity compared to the untreated control group. The potency percentage of the analgesic activity of tested compounds ranged from 3.61% with compound **5c** to

35.90 % with compound **10** after 90 min, with no significant difference from piroxicam (39.71%). Moreover, it is being found that compound **10** possessed most rapid and sustained duration antinociceptive activity till 90 min. This may be due to 4-fluorophenyl moiety were incorporated to pyranopyrazole, or /and pyrazolopyranopyrimidine were found to exhibit good analgesic activity[17]. On the other hand, compounds showed **4c**, **7b** and **7c** moderate antinociceptive activity comparable to standard piroxicam positive control. The data was represented in table 3,4.

Table 3: Analgesic evaluation of tested compounds.

Groups	% Change from baseline		
	30 min	60 min	90 min
Control	11.59 ± 2.846	11.68 ± 0.460	6.966 ± 0.023
Piroxicam	64.41 ± 3.721	191.6 ± 20.510*	283.6 ± 12.236*
4a	138.9 ± 13.341*	156.9 ± 7.896	174.9 ± 12.365
4b	41.61 ± 5.246	82.50 ± 8.965	69.12 ± 7.265
4c	85.15 ± 3.905	255.4 ± 12.398*	228.7 ± 19.856*
5a	17.50 ± 1.0123	157.8 ± 13.269	161.5 ± 13.365
5b	74.66 ± 6.987	108.1 ± 9.867	132.0 ± 10.236
5c	17.74 ± 0.112	41.90 ± 2.884	32.16 ± 2.365
6a	105.7 ± 12.31*	88.21 ± 6.598	78.16 ± 1.263
6b	26.91 ± 1.236	37.55 ± 4.981	55.86 ± 4.965
7a	37.02 ± 2.658	77.61 ± 6.588	110.6 ± 12.365
7b	89.12 ± 9.855	160.6 ± 7.896	243.7 ± 20.365
7c	129.5 ± 11.236*	143.1 ± 10.236	216.7 ± 18.365
8	69.85 ± 5.687	81.64 ± 12.27	108.6 ± 5.62
9	49.87 ± 1.234	100.9 ± 7.607	138.0 ± 4.123
10	56.60 ± 6.923	222.0 ± 12.365*	257.1 ± 23.365*

P < 0.05: Statistically significant from Control. (One way Anova followed by Tukey test).

Table 4: % Potency of tested compounds after 30, 60 and 90 min.

Group	Potency from control		
	30 min	60 min	90 min
Piroxicam	45.570	15.404	39.712
4a	10.98447	12.43322	24.10767
4b	2.590164	6.063356	8.922481
4c	6.346851	20.86644	31.83089
5a	0.509922	12.51027	22.18404
5b	5.44176	8.255137	17.94918
5c	0.53063	2.587329	3.61671
6a	8.119931	6.552226	10.22021
6b	1.321829	2.214897	7.018949
7a	2.194133	5.644692	14.87712
7b	6.689387	12.75	33.98421
7c	10.17343	11.25171	30.10824
8	5.026747	5.989726	14.59001
9	3.302847	7.638699	18.81051
10	3.88352	18.00685	35.90784

3.2.3. Results of antipyretic screening

Antipyretic activity of the novel compounds was evaluated following The subcutaneous injection of Brewer's yeast suspension method at a dose of one ml/100 g body weight of 44%. In this method the antipyretic activity was administered by an intramuscular injection into each animal of all the tested groups. The site of injection was then massaged to spread the suspension into the tissues. Before yeast injection rectal temperature was recorded for all groups. The rectal temperature measured 18 hours following the yeast injection serves as the basic line of elevated body temperature, to which the anti-pyretic effect will be compared. At that specific time (18

hours after yeast injection) drugs were administered. As shown in table 5, compounds **4b** and **5c** showed a significant antipyretic activity compared to the untreated control group. The potency percentage of the antipyretic activity of tested compounds ranged from **35.56±0.160** with compound **5a** to **36.20±0.167** with compound **4b** after 90 min., with no significant difference from piroxicam **35.80±0.207**. Moreover, it is being found that compound **4b** and **5c** possessed excellent activity till 2 h post yeast. On the other hand, compounds **4a**, **5b**, **6a**, **6b** and **9** showed antipyretic activity similar to standard piroxicam positive control. The data was represented in table 5.

Table 5: Antipyretic effects of the tested compounds.

Group	Baseline	Elevated temperature	1 h post yeast	2 h post yeast
Control	35.86±0.192	36.90±0.089	36.82±0.131	36.94±0.128
Paracetamol	35.66±0.2015	36.34±0.233	35.82±0.198*	35.80±0.207*
4a	35.64±0.2015	36.60±0.192	35.96±0.215*	35.74±0.140*
4b	35.62±0.1463	37.06±0.222	36.64±0.092	36.20±0.167
5a	35.66±0.2015	36.30±0.216	35.96±0.188*	35.56±0.160*
5b	35.66±0.2005	36.96±0.081	36.54±0.097	35.92±0.196*
5c	35.66±0.2011	36.10±0.187	36.04±0.163	36.08±0.115*
6a	35.66±0.2011	36.98±0.037	36.54±0.116	35.86±0.132*
6b	35.66±0.2015	36.98±0.031	36.48±0.177	35.82±0.111*
9	35.90±0.2236	36.38±0.269	36.10±0.192	35.76±0.050*

The data represents the mean ± standard error of the mean (n = 6). Values represent the mean ± S.E. of six animals for each groups. * P<0.05: Statistically significant from Control. (One way Anova followed by Tukey test).

3.2.4. Results of gastric ulcerative effect

Compounds with significant anti-inflammatory profile were tested for GI-ulcerogenicity potential. The ulcerative effect of test compounds has been inspected visually relative to the known ulcerogenic drug, piroxicam. After gross visual inspection, it has been obvious that all compounds showed no ulcer formation, whereas piroxicam

showed significant ulcerogenic effect. The data was represented in table 6.

4. Conclusion

The data revealed that, the investigation of anti-inflammatory screening of all prepared compounds showed marked anti-inflammatory properties. Compound **9** possessed the highest anti-inflammatory activity in this work. Other compounds exhibited comparable activity to the reference drug

piroxicam. The fused pyranopyrimidine system accompanied with pyrazole moiety resulted in potentiating of the anti-inflammatory effect. On the other hand, compounds **4c**, **7b**, **7c** and **10** recorded equipotent antalgic activity compared to standard drug and compounds **4b** and **5c** showed a significant analgesic activity. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds.

Fortunately, the test for the ulcerogenic activity revealed that none of the new compounds possess a significant ulcer-inducing activity, a property that qualifies these compounds for further pre-clinical investigation.

Table 6: Effect on stomach:

Group	Ulcer number	Ulcer severity
Control	-	-
Piroxicam	11.8±0.985*	22.4±1.652*
4a	-	-
4b	-	-
4c	-	-
5a	-	-
5b	-	-
5c	-	-
6a	-	-
6b	-	-
7a	-	-
7b	-	-
7c	-	-
8	-	-
9	-	-
10	-	-

All tested drugs showed significant anti-inflammatory effects.

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10/12/2014