#### Design and Synthesis of novel pyrano[2,3-c]pyrazoles and related fused ring systems and evaluation of antiinflammatory, analgesic and antipyretic activities.

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**Abstract:** A series of some new pyrano[2,3-*d*]pyrimidine derivatives were synthesized and evaluated for their antiinflammatory as well as analgesic and antipyretic activities. The results showed that all compounds possessed promising anti-inflammatory activity. Compounds **6a** and **9b** have shown a potent anti-inflammatory activity more than piroxicam reference drug. Whereas, compounds **6c**, **8a**, **9a** and **10a**,**b** exhibited equipotent analgesic activity compared to piroxicam and compound **10b** showed excellent antipyretic activity more than piroxicam. None of the tested compounds showed an ulcerogenic effect.

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#### 1. Introduction

Currently available non-steroidal antiinflammatory drugs (NSAIDs) remain widely prescribed medications worldwide for the treatment of pain, fever and swelling associated with arthritis [1,2]. Most of them 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 cycloxygenases-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®). Fig.1.[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 selective COX2 anti-inflammatory agents

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



structure in medicinal chemistry. In the current work, the challenge was to synthesize novel derivatives of pyrazolopyranopyrimidine to maintain sufficient efficacies in human pain models and to reduce side effects. Compounds **1** and **2** (Fig.2).



Fig. 2. The designed target compounds

## 2. Experimental protocols

## 2.1. General remarks:

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<sup>-</sup> <sup>1</sup>H-NMR and <sup>13</sup>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  $\delta$  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 chromatoghraphy (TLC) on silica gel olates 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 ±0.4%. 6-Amino-3-methyl-1-phenyl-4-(4-substituted phenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-

carbonitrile  $(\mathbf{3}_{a-c})$ , were prepared as reported procedures [18].

## 2.2. Chemistry

General procedure for the synthesis of: 3-Methyl-5-oxo-1-phenyl-4-(substitutedphenyl)-1,4,5,6tetrahydropyrazolo[4',3':5,6]pyrano[2,3d]pyrimidine-7-carbonyl chloride (4<sub>a-c</sub>) And 3-Methyl-1-phenyl-4-(substitutedphenyl)-4,6dihydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidine-5(1H)-one-7-carbonyl chloride (5<sub>a,b</sub>).

A mixture of compounds  $\mathbf{3}_{a-c}$  (0.01 mol) in dry benzene (20 mL), anhydrous potassium carbonate (1.4 g, 0.01 mol) and either oxalyl chloride (1.9 g, 1.3 mL, 0.015 mol) or chloroacetyl chloride (1.6 g, 1.2 mL, 0.015 mol) was added drop wise for 30 min. at 10 °C. The reaction mixture was stirred for 55 hours at room temperature. The excess solvent was evaporated under reduced pressure till dryness and cooled. The formed residue was washed twice with water (10 mL), dried and crystallized from acetonitrile.

## 3-Methyl-5-oxo-1,4-diphenyl-1,4,5,6tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidine-7-carbonyl chloride (4<sub>a</sub>)

Yellow powder; yield 67%; mp: 164-166 °C; IR (KBr, cm<sup>-1</sup>): 3429 (NH), 2920 (CH aliphatic), 1714 (CO-Cl), 1663 (C=O of amide); <sup>1</sup>H-NMR (DMSO $d_6$ ):  $\delta$  1.91 (s, 3H, CH<sub>3</sub>), 5.07 (s, 1H, pyrano), 7.16-7.76 (m, 10H, ArH), 12.65 (s, 1H, NH exchangeable by D<sub>2</sub>O); MS (EI) m/z: 418.20 (M<sup>+</sup>, 58.70%), 278.90 (100%); Anal. Calcd for C<sub>22</sub>H<sub>15</sub>ClN<sub>4</sub>O<sub>3</sub> (418.83): C, 63.09; H, 3.61.; N, 13.38 Found C, 63.43; H, 3.41; N, 13.75.

## 4-(4-Chlorophenyl)-3-methyl-5-oxo-1-phenyl-1,4,5,6-tetrahydropyrazolo [4',3':5,6]pyrano[2,3*d*]pyrimidine-7-carbonyl chloride (4<sub>b</sub>)

Pale yellow powder; yield 66%; mp: 150-152 °C; IR (KBr, cm<sup>-1</sup>) : 3367 (NH), 2924 (CH aliphatic), 1735 (CO-Cl), 1685 (C=O of amide) ;<sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.92 (s, 3H, CH<sub>3</sub>), 5.14 (s, 1H, pyrano), 7.35-7.44 (m, 9H, ArH), 12.01 (s, 1H, NH exchangeable by D<sub>2</sub>O) ; MS (EI) m/z: 450.10 (M-2, 1.48%), 388.15 (100%); Anal. Calcd for C<sub>22</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>3</sub> (453.28): C, 58.29; H, 3.11; N, 12.36. Found C, 58.17; H, 3.19; N, 12.68.

4-(4-Bromophenyl)-3-methyl-5-oxo-1-phenyl-

## 1,4,5,6-tetrahydropyrazolo[4',3':5,6] pyrano[2,3*d*]pyrimidine-7-carbonyl chloride (4<sub>c</sub>)

Orange powder; yield 80%; mp: 146-148 °C; IR (KBr, cm<sup>-1</sup>): 3262 (NH), 2923 (CH aliphatic), 1690 (CO-Cl), 1684 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.91 (s, 3H, CH<sub>3</sub>), 5.17 (s, 1H, pyrano), 7.28-7.74 (m, 9H, ArH), 8.51(s, 1H, NH exchangeable by D<sub>2</sub>O);

Anal. Calcd for  $C_{22}H_{14}BrClN_4O_3$  (497.73): C, 53.09; H, 2.84; N, 11.26. Found : C , 53.32; H, 2.95; N, 11.51

#### 7-Chloromethyl-3-methyl-1,4-diphenyl-4,6dihydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidine-5(1*H*)-one (5<sub>2</sub>)

Yellow powder; yield 74%; mp: 144-146 °C; IR (KBr, cm<sup>-1</sup>): 3182 (NH), 2924 (CH aliphatic), 1681 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$ 1.90 (s, 3H, CH<sub>3</sub>), 4.34 (s, 2H, CH<sub>2</sub>Cl), 4.49 (s, 1H, pyrano), 7.10-7.71 (m, 10H, ArH), 7.99 (s, 1H, NH exchangeable by D<sub>2</sub>O); MS (EI) m/z: 404.90 (M+H, 43.9%), 406.45 (M+2, 42.76%), 152.90 (100%); Anal. Calc for C<sub>22</sub>H<sub>17</sub>ClN<sub>4</sub>O<sub>2</sub> (404.84): C, 65.27; H, 4.23; N, 13.84. Found: C, 65.39; H, 4.38; N, 14.20.

#### 4-(4-Bromophenyl)-7-chloromethyl-3-methyl-1phenyl-1,4-dihydropyrazolo[4',3':5,6] pyrano[2,3*d*]pyrimidin-5(1*H*)-one (5<sub>b</sub>)

Orange powder; yield 79%; mp: 127-129 °C; IR (KBr, cm<sup>-1</sup>): 3170 (NH), 2931 (CH aliphatic), 1685 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  2 .21 (s, 3H, CH<sub>3</sub>), 4.37 (s, 2H, CH<sub>2</sub>Cl), 4.90 (s, 1H, pyrano), 7.80, 7.77 (2d, 4H, *J*=8.4 Hz, ArH), 7.17-8.15 (m, 5H, ArH), 8.01 (s, 1H, NH, exchangeable by D<sub>2</sub>O); Anal. Calc for C<sub>22</sub>H<sub>16</sub>BrClN<sub>4</sub>O<sub>2</sub> (483.75): C, 54.62; H, 3.33; N, 11.58. Found C, 54.81; H, 3.29; N, 11.87.

General procedure for the synthesis of: *N*-(4-Chlorophenyl)-3-methyl-5-oxo-1-phenyl-4-(4-substitutedphenyl)-1.4.5.6-

tetrahydroPyrazolo[4',3':5,6]pyrano[2,3-

*d*]pyrimidine-7-carboxamide(6<sub>a-c</sub>), 3-Methyl-5-oxo-1-phenyl-*N*-{4-[(pyrimidin-2-ylamino)sulfonyl] phenyl}-4-(4-substitutedphenyl)-1,4,5,6-

tetrahydropyrazolo(4',3':5,6)pyrano(2,3-

*d*)pyrimidine-7-carboxamide( $7_{a-c}$ ), 7-{[(4-

Chlorophenyl)amino]methyl}-3-methyl-1-phenyl-4-(substitutedphenyl)-4,6-

dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (8<sub>a,b</sub>) and 4-{[(3-Methyl-5-oxo-1-phenyl-4-(4-substitutedphenyl)-1,4,5,6-

tetrahydropyrazolo[4',3':5,6]pyrano[2,3-

## *d*]pyrimidin-7-yl)methyl]amino}-*N*-pyrimidin -2ylbenzenesulfonamide(9<sub>a,b</sub>).

A mixture of compounds  $\mathbf{4}_{a-c}$  or  $\mathbf{5}_{a-c}$  (0.01mol) in dry benzene (20 mL), anhydrous potassium carbonate (1.4 g, 0.01mol) and either p-chloroaniline (1.2 g, 0.01mol) or sulfadiazine (2.5 g, 0.01mol) was added. The reaction mixture was heated under reflux for 10 hours, the excess solvent was evaporated under reduced pressure to half its volume and cooled. The formed solid was filtered, washed twice with water (15 mL), dried and crystallized from ethanol.

## $\label{eq:N-(4-Chlorophenyl)-3-methyl-5-oxo-1,4-diphenyl-1,4,5,6-dihydropyrazolo$

[4',3':5,6]pyrano[2,3-*d*]pyrimidin-7-carboxamide (6<sub>a</sub>) Brown micro crystals; yield 40%; mp: 162-164 °C; IR (KBr, cm<sup>-1</sup>): 3356 (NH), 2871 (CH aliphatic), 1675, 1651 (2C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$ 1.91 (s, 3H, CH<sub>3</sub>), 5.07 (s, 1H, pyrano), 7.19-7.88 (m, 10H, ArH), 7.43, 7.91 (2d, 4H, *J*=9 Hz, ArH), 11.02, 12.80 (2s, 2H, NH exchangeable by D<sub>2</sub>O); Anal. Calcd for C<sub>28</sub>H<sub>20</sub>ClN<sub>5</sub>O<sub>3</sub> (509.93): C; 65.95, H; 3.95, N; 13.73. Found: C; 66.13, H; 4.09, N; 13.97.

### *N*-4-bis(4-Chlorophenyl)-3-methyl-5-oxo-1-phenyl-1,4,5,6-tetrahydropyrazolo [4',3':5,6]pyrano[2,3*d*]pyrimidine-7-carboxamide (6<sub>b</sub>)

Red micro crystals; yield 36%; mp: 130-132 °C; IR (KBr, cm<sup>-1</sup>): 3350 (NH), 2904 (CH aliphatic), 1668, 1653 (2C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.93 (s, 3H, CH<sub>3</sub>), 5.20 (s, 1H, pyrano), 7.28-7.69 (m, 9H, ArH), 7.63, 7.72 (2d, 4H, *J*=8.1 Hz, ArH), 9.29, 9.61 (2s, 2H, NH exchangeable by D<sub>2</sub>O); <sup>13</sup>C-NMR (DMSO): 13.06, 34.93, 100.13, 104.59, 115.75, 120.86(2C), 125.73(2C), 128.47(2C), 129.02, 129.20, 129.33(2C), 129.49, 129.68, 129.88(2C), 138.05, 138.33, 142.33, 146.18, 146.68, 148.18, 149.88, 157.57, 161.32; Anal. Calcd for C<sub>28</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>3</sub> (544.39): C, 61.78; H, 3.52; N, 12.86. Found C, 61.92; H, 3.60; N, 13.16.

## 4-(4-Bromophenyl)-*N*-(4-chlorophenyl)-3-methyl-5-oxo-1-phenyl-1,4,5,6-tetrahydro

## pyrazolo[4',3':5,6]pyrano(2,3-*d*)pyrimidine-7carboxamide (6<sub>c</sub>)

Buff powder; yield 25%; mp: 120-122 °C; IR (KBr, cm<sup>-1</sup>): 3421( NH), 2920 (CH aliphatic), 1670, 1660 (2 C=O); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.94 (s, 3H, CH<sub>3</sub>), 5.13 (s, 1H, pyrano), 7.48, 7.91 (2d, 4H, J=7.2 Hz, ArH), 7.27-7.83 (m, 9H, ArH), 10.98, 11.01 (2s, 2H, NH exchangeable by D<sub>2</sub>O); MS (EI) m/z: 588.00 (M+H, 35.47%), 589.00 (M+2.)27.92%), 54.00 (100%); Anal. Calcd for C<sub>28</sub>H<sub>19</sub>BrClN<sub>5</sub>O<sub>3</sub> (588.83): C, 57.11; H, 3.25; N, 11.89. Found: C, 57.28; H, 3.51; N, 12.23.

# 3-Methyl-5-oxo-1,4-diphenyl-N-{4-[(pyrimidin-2-ylamino)sulfonyl]phenyl}-1,4,5,6-

## tetrahydropyrazolo[4',3':5,6]pyrano[2,3-

*d*]pyrimidine-7-carboxamide (7<sub>a</sub>)

White needle crystals; yield 32%; mp: 260-262 °C; IR (KBr, cm<sup>-1</sup>): 3356 (NH), 2926 (CH aliphatic) 1678, 1653 (2C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ) :  $\delta$ 1.91 (s, 3H, CH<sub>3</sub>), 5.16 (s, 1H, pyrano), 6.54 (d, 2H, ArH), 6.99-7.52 (m, 9H, ArH), 7.55,7.79 (2d, 4H, *J*=9 Hz, ArH), 7.99 (s, 2H, NH, exchangeable by D<sub>2</sub>O), 8.49 (d, 2H, ArH), 11.14, (s, 1H, NH, exchangeable by D<sub>2</sub>O); MS (EI) m/z: 632.0 (M<sup>+</sup>, 89.52%), 634.0 (M+2, 89.52%), 635.0 (M+3, 70.48%), 95.00 (100%); Anal. Calcd for C<sub>32</sub>H<sub>24</sub>N<sub>8</sub>O<sub>5</sub>S (632.65) : C, 60.75; H, 3.82; N, 17.71. Found: C, 60.81; H, 3.93; N, 17.94.

4-(4-Chlorophenyl)-3-methyl-5-oxo-1-phenyl-*N*-{4-[(pyrimidin-2-ylamino)sulfonyl]

## phenyl}-1,4,5,6-tetrahydropyrazolo [4',3':5,6] pyrano [2,3-d] pyrimidine-7-carboxamide (7<sub>b</sub>)

Brown needle crystals; yield 45%; mp: 206-208°C; IR (KBr, cm<sup>-1</sup>): 3354 (NH), 2904 (CH aliphatic), 1675, 1653 (2C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ) :  $\delta$  1.90 (s, 3H, CH<sub>3</sub>), 4.93 (s, 1H, pyrano), 7.14, 7.37 (2d, 4H, *J*=7.8 Hz, ArH), 7.05-7.81 (m, 6H, ArH), 7.91, 7.88 (2d, 4H, *J*=9 Hz, ArH), 8.02 (d, 2H, ArH), 8.63, 8.91, 10.00 (3s, 3H, NH exchangeable by D<sub>2</sub>O); Anal. Calcd for C<sub>32</sub>H<sub>23</sub>ClN<sub>8</sub>O<sub>5</sub>S (667.09): C, 57.61; H, 3.48; N, 16.80. Found: C, 57.73; H, 3.53; N, 17.02.

#### 4-(4-Bromophenyl)-3-methyl-5-oxo-1-phenyl-N-{4-[(pyrimidin-2-ylamino)sulfonyl]phenyl}-1,4,5,6tetrahydropyrazolo[4',3':5,6]pyrano(2,3*d*)pyrimidine-7-carboxamide (7<sub>c</sub>)

White needle crystals; yield 77%; mp: 218-220°C; IR (KBr, cm<sup>-1</sup>): 3354 (NH), 2922 (CH aliphatic), 1697, 1653 (2C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.92 (s, 3H, CH<sub>3</sub>), 5.11 (s, 1H, pyrano), 6.99-7.33 (m, 6H, ArH), 6.55, 7.59 (2d, 4H, *J*=8.7 Hz, ArH), 7.63, 8.46 (2d, 4H, ArH), 8.49 (d, 2H, ArH), 4.38, 6.02, 11.27 (3s, 3H, NH exchangeable by D<sub>2</sub>O); Anal. Calcd for C<sub>32</sub>H<sub>23</sub>BrN<sub>8</sub>O<sub>5</sub>S (711.54 ): C, 54.02; H, 3.26; N, 15.75. Found: C, 54.11; H; 3.31; N, 15.93.

## 7-{[(4-Chlorophenyl)amino]methyl}-3-methyl-1,4diphenyl-4,6-dihydropyrazolo

## [4',3':5,6]pyrano[2,3-d]pyrimidin-5(1*H*)- one (8<sub>a</sub>)

Brown micro crystals; yield 61%; mp: 290-292 °C; IR (KBr, cm<sup>-1</sup>): 3500 (NH), 2897 (CH aliphatic), 1650 (C=O of amide); <sup>1</sup>HNMR (DMSO- $d_6$ ):  $\delta$  2.22 (s, 3H, CH<sub>3</sub>), 3.85 (s, 2H, CH<sub>2</sub>-NH), 4.96 (s, 1H, pyrano), 7.31, 7.71 (2d, 4H, ArH), 6.54-7.61 (m, 10H, ArH), 9.33, 10.51 (2s, 2H, NH, exchangeable by D<sub>2</sub>O); Anal. Calcd for C<sub>28</sub>H<sub>22</sub>ClN<sub>5</sub>O<sub>2</sub> (495.14): C, 67.81; H, 4.47; N, 14.12. Found: C, 68.05; H, 4.54; N, 14.43.

## 4-(4-Bromophenyl)-7-{[(4-

## chlorophenyl)amino]methyl}-3-methyl-1-phenyl-4,6-dihydro

#### pyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidin-5(1*H*)one (8<sub>b</sub>)

White crystals; yield 52%; mp: 130-132 °C; IR (KBr, cm<sup>-1</sup>) : 3397 (NH), 2919 (CH aliphatic), 1692 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ) :  $\delta$  1.89 (s, 3H, CH<sub>3</sub>), 4.19 (s, 2H, CH<sub>2</sub>-NH) 4.89, (s, 1H, pyrano), 7.18-7.80 (m, 5H, ArH), 7.36, 8.52 (2d, 4H, *J*=8.7 Hz, ArH), 7.44, 7.71 (2d, 4H, *J*=6 Hz, ArH), 8.55, 9.34 (2s, 2H, NH, exchangeable by D<sub>2</sub>O); MS (EI) m/z: 573.00 (M<sup>+</sup>, 58.56%), 574.00 (M+H, 68.47), 515.00 (100%); Anal. Calcd for C<sub>28</sub>H<sub>21</sub>BrClN<sub>5</sub>O<sub>2</sub> (574.85): C, 58.50; H, 3.68; N, 12.18. Found : C, 58.63; H, 3.81; N, 12.42. **4-{[(3-Methyl-5-oxo-1,4-diphenyl-1,4,5,6-**

tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d] pyrimidin-7-yl)methyl]amino}-N-pyrimidin-2-

ylbenzenesulfonamide  $(9_a)$ 

White needle crystals; yield; 81%; mp: 256-258 °C; I R (KBr, cm<sup>-1</sup>): 3433 (NH), 2808 (CH aliphatic), 1649 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.90 (s, 3H, CH<sub>3</sub>), 4.20 (s, 2H, CH<sub>2</sub>.NH), 5.01 (s, 1H, pyrano), 7.23-7.72 (m, 12H, ArH), 7.26, 7.72 (2d, 4H, J=9 Hz, ArH), 7.76 (d, 2H, ArH), 12.64 (s, 2H, NH exchangeable by  $D_2O$ ; <sup>13</sup>C-NMR (DMSO): 13.84, 35.86, 60.02, 106.28, 117.77, 118.27(2C), 119.09, 120.75(2C), 121.78, 124.38(2C), 125.72, 128.04(2C), 129.03(2C), 130.03(2C), 132.47, 136.11, 137.08, 143.28, 147.20, 157.31(2C), 161.11, 161.96, 168.41, 171.02, 173.80; MS (EI) m/z: 618.10 (M<sup>+</sup>, 3.03%), 619.10 (M+H, 2.02%), 92.10 (100%); Anal. Calcd for C<sub>32</sub>H<sub>26</sub>N<sub>8</sub>O<sub>4</sub>S (618.66): C, 62.12; H, 4.24; N, 18. 11. Found: C, 62.32; H, 4.29; N, 18.42 4-({[4-(4-Bromophenyl)-3-methyl-5-oxo-1-phenyl-1,4,5,6-tetrahydropyrazolo[4',3':5,6]

#### pyrano[2,3-*d*]pyrimidin-7-yl]methyl}amino)-*N*pyrimidin-2-ylbenzenesulfonamide (9<sub>b</sub>)

White needle crystals; yield 64%; mp: 253-255 °C; IR (KBR cm<sup>-1</sup>) : 3421 (NH), 2890 (CH aliphatic), 1651 (C=O of amide); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.90 (s, 3H, CH<sub>3</sub>), 4.94 (s, 2H, CH<sub>2</sub>-NH), 5.20 (s, 1H, pyran), 7.21-7.91 (m, 6H, ArH), 7.47, 7.64 (2d, 4H, *J*=7.8 Hz, ArH), 7.32, 7.88 (d, 4H, ArH), 8.54 (d, 2H, ArH), 10,01, 13.91 (2s, 3H, NH, exchangeable by D<sub>2</sub>O); Anal. Calcd for C<sub>32</sub>H<sub>25</sub>BrN<sub>8</sub>O<sub>4</sub>S (697.56): C, 55.10; H, 3.61; N, 16.06. Found: C, 55.38; H, 3.82; N, 16.39.

General procedure for the synthesis of: 5-Amino-3methyl-1,6-diphenyl-4-(4-substitutedphenyl)-4,8dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-

7(1H)-thione  $(10_{a,b})$ 

A solution of compounds  $\mathbf{3}_{a,b}$  (0.01mol) in dimethylformamide (30 mL), phenylisothiocyanate (1.5g, 0.01mol) and triethylamine (1mL), was heated under reflux for 20 hours. The reaction mixture was cooled and poured into ice-cold water (20 mL). The formed precipitate was filtered, dried and crystallized from methanol.

5-Amino-4-(4-chlorophenyl)-3-methyl-1,6-diphenyl-4,8-dihydropyrazolo[4',3':5,6]pyrano [2,3*d*]pyrimidine-7(1*H*)-thione (10<sub>a</sub>)

Dark brown micro crystals; yield 30 %, mp: 183-185° C; IR (KBr, cm<sup>-1</sup>): 3417 (NH<sub>2</sub>), 2900 (CH aliphatic), 1373 (C=S); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$ 2.13 (s, 3H, CH<sub>3</sub>), 2.55 (s, 2H, NH<sub>2</sub> exchangeable by D<sub>2</sub>O), 4.02 (s, 1H, pyrano), 7.09-7.98 (m, 10H, ArH), 7.12, 7.64 (2d, 4H, ArH); <sup>13</sup>C-NMR (DMSO): 11.61, 30.73, 92.00, 102.00, 117.55 (2C), 118.14, 121.74 (2C), 123.67 (2C), 125.29, 126.00, 128.69 (2C), 129.31 (2C), 129.58 (2C), 129.98, 137.90, 138.00, 146.00, 150.00, 160.00, 162.22, 167.00; Anal. Calcd for C<sub>27</sub>H<sub>22</sub>ClN<sub>5</sub>OS (500.01): C, 64.86; H, 4.43; N, 14.01. Found: C, 64.98; H, 4.22; N, 14.12. 5-Amino-4-(4-bromophenyl)-3-methyl-1,6-

diphenyl-4,8-dihydropyrazolo[4',3':5,6]pyrano [2,3-d]pyrimidine-7(1*H*)- thione (10<sub>b</sub>)

Brown micro crystals; yield 40%; mp: 90-92°C; IR (KBr, cm<sup>-1</sup>): 3411 (NH<sub>2</sub>), 2921 (CH aliphatic), 1370 (C=S); <sup>1</sup>H-NMR (DMSO- $d_6$ ) :  $\delta$  2.28 (s, 3H, CH<sub>3</sub>), 2.56 (s, 2H, NH<sub>2</sub> exchangeable by D<sub>2</sub>O), 3.35 (s, 1H, pyrano), 6.99-7.59 (m, 10H, ArH), 7.15, 7.98 (2d, 4H, ArH); MS (EI) m/z: 544.10 (M+H, 17.17%), 93.15 (100%); Anal. Calcd for C<sub>27</sub>H<sub>22</sub>BrN<sub>5</sub>OS (544.47): C, 59.56; H, 4.07; N, 12.86. Found: C, 59.61; H, 4.17; N, 13.1.

## General procedure for the synthesis of: 3-Methyl-1-phenyl-4-(4-substitutedphenyl)-6,8-

## dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5,7-(1H,4H)-dithione $(11_{a,b})$

A suspension of compounds  $\mathbf{3}_{a,b}$  (0.01 mol) in dry pyridine (20 mL) and carbon disulphide (1g, 0.01 mol), was heated under reflux for 36 hours. The reaction mixture was cooled, poured into ice-cold water (20 mL) and neutralized with 10% diluted hydrochloric till complete precipitation. The formed precipitate was filtered, dried and crystallized from methanol.

#### 4-(4-Chlorophenyl)-3-methyl-1-phenyl-6,8dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*] pyrimidine-5,7(1*H*,4*H*)-dithione (11<sub>a</sub>)

Red micro crystals; yield 55 %; mp: 150-152°C; IR (KBr, cm<sup>-1</sup>): 3421 (NH<sub>2</sub>), 2916 (CH aliphatic), 1363 (C=S); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.90 (s, 3H, CH<sub>3</sub>), 4.96 (s, 1H, pyrano), 7.02-7.91 (m, 5H, ArH), 7.33, 7.67 (2d, 4H, J=9Hz, ArH), 10.01, 13.92 (2s, 2H, NH exchangeable by D<sub>2</sub>O); MS (EI) m/z: 440.30 (M+2, 28.25%), 62.10 (100%); Anal. Calcd for C<sub>21</sub>H<sub>15</sub>ClN<sub>4</sub>OS<sub>2</sub> (438.95): C, 57.46; H, 3.44; N, 12.76 Found C, 57.54; H, 3.51; N, 12.93.

## 4-(4-Bromophenyl)-3-methyl-1-phenyl-6,8dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*] pyrimidine-5,7-(1*H*,4*H*)-dithione (11<sub>b</sub>)

Yellow micro crystals; yield 58%; mp: 125-127°C; IR (KBr, cm<sup>-1</sup>): 3407 (NH<sub>2</sub>), 2926 (CH aliphatic), 1371 (C=S); <sup>1</sup>H-NMR (DMSO- $d_6$ ):  $\delta$  1.91 (s, 3H, CH<sub>3</sub>), 4.94 (s, 1H, pyrano), 7.22-7.87 (m, 5H, ArH), 7.46, 7.83 (2d, 4H, *J*=7.5 Hz, ArH), 9.61, 10.01 (s, 2H, NH exchangeable by D<sub>2</sub>O); <sup>13</sup>C-NMR (DMSO) 12.09, 40.33, 104.97, 119.65, 121.16, 126.24, 129.25 (2C), 130.83 (2C), 131.07, 131.48 (2C), 132.28 (2C), 137.67, 142.03, 146.66, 172.61, 173.50, 192.76; Anal .Calcd for C<sub>21</sub>H<sub>15</sub>BrN<sub>4</sub>OS<sub>2</sub> (483.40): C, 52.18; H, 3.13; N, 11.59. Found: C, 52.32; H, 3.18; N, 11.78.

## 2.3. Biological evaluation

## **2.3.1.** 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: carrageenean) into the plantar tissue of the paw following to Winter method [19].

## 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 6a-c to 11a,b 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 hrs 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  $20 \text{ mg kg}^{-1}[20]$ , (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) [21]. Each value represents the mean  $\pm$  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).

## 2.3.2. Analgesic activity evaluation

## Procedure

Antinociceptive responses were determined using the tail-flick test [22]. 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<sup>-1</sup>. The data represents the mean  $\pm$  standard error of the mean (n = 6). Values represent the mean  $\pm$ S.E. of six animals for each groups.

## 2.3.3. 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 [Roszkowski *et al.*, 1971]

#### 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 [Roszkowski et al., 1971]. Rectal temperature was recorded by a multichannel electric thermometer (TMP 812 Digital Thermometer, Ugo Basile, Comerio, Italy) 1 and 2 hrs after administration of drugs [Panthong et al., 2003]. The increase in rectal temperature at different times in respect to the values before administration of the yeast was calculated.

## **2.3.4.** Ulcerogenic effect in rats Procedure

Acute ulcerogenicity was determined according to the method of Szelenyi [Szelenyi and Thiemer, 1978]. 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) [23,24]. 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  $11_{a,b}$  was shown in Schemes 1&2. Starting compounds  $\mathbf{3}_{a-c}$  were prepared in 82-96% yields by reaction of 3-methyl-1phenyl-5-pyrazolone (1) commercially available and appropriate arylidinemalononitrile (2) as reported procedure [25]. The intermediate chloro compounds  $\mathbf{4}_{\mathbf{a}-\mathbf{c}}$  and  $\mathbf{5}_{\mathbf{a},\mathbf{b}}$  were synthesized through the reaction of  $\mathbf{3}_{a-c}$  with appropriate acid chloride under anhydrous condition. The IR spectra of compounds  $4_{a-c}$  showed disappearance of absorption bands for (NH<sub>2</sub>) and appearance of additional absorption bands in the range of 1690-1735cm<sup>-1</sup> which confirmed the presence of carbonyl function (COCl). Furthermore, the <sup>1</sup>H-NMR spectra of  $5_{a,b}$  showed a singlet signal for CH<sub>2</sub>Cl at 4.34 and 4.37 ppm, respectively. On the other hand, synthesis of target compounds  $6_{a-c}$ ,  $7_{a-c}$ ,  $8_{a,b}$  and  $9_{a,b}$ were achieved by heating appropriate amines, namely, p-chloroaniline and / or sulfadiazine and the corresponding chloro precursor compounds  $4_{a-c}$  and  $\mathbf{5}_{a,b}$  in boiling benzene [26]. The structures of all novel synthesized compounds were determined by spectral and microanalytical analyses. The <sup>1</sup>H-NMR spectra of compounds  $\mathbf{6}_{\mathbf{a}-\mathbf{c}}$  -  $\mathbf{9}_{\mathbf{a},\mathbf{b}}$  have shown an additional peaks around  $\delta$  6.54-8.54 ppm corresponding to the aromatic protons, in addition to NH signals at  $\delta$  4.38-13.91 ppm exchangeable protons by D<sub>2</sub>O.

Moreover, in Scheme 2 compounds  $10_{a,b}$  and  $11_{a,b}$  were prepared by reaction of  $3_{a,b}$  either with phenylisothiocyante or carbon disulphide in basic medium [27]. The IR spectra exhibited the expected bands for the characteristic groups as NH stretching vibration, CH<sub>3</sub> stretching and another specific band for thiocarbonyl C=S vibration which are present in the compounds  $10_{a,b}$  and  $11_{a,b}$ . The <sup>1</sup>H-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



#### **3.2.** Pharmacological evaluation

Pharmacological screening of novel synthesized compounds was carried-out at the National Research Centre, Pharmacology Department, Egypt. Antiinflammatory activities of all novel synthesized compounds were assessed by utilizing carrageenaninduced rat's paw edema model [19-21], while analgesic activities were investigated by Tail Flick test [22]. Additionally, antipyretic activities were achieved by the subcutaneous injection of Brewer's yeast suspension [Roszkowski *et al.*, 1971 and Panthong *et al.*, 2003] and acute ulcerogenicity was evaluated relative to piroxicam drug as positive control [24,25] and the obtained results of the tested pyranopyrimidines and their analogues are shown in Tables 1- 6.

## **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<sup>-1</sup> compared to reference drug piroxicam (20 mg kg<sup>-1</sup>). The relative percentage inhibition of edema recorded values around 80.71% (compound **6a**) to 43.10% (compound **10b**),

while the reference standard showed 62.17% inhibition of edema after four hours. Compound 6a possessed an excellent anti-inflammatory activity with potency value 80.71% after four hours. This may be attributed to the unsubstituted aromatic ring at 4-position, in addition to exocyclic carbonyl group at 7-position gave rise to an increased anti-inflammatory activity compared to other related congener [28-30]. Additionally, compounds 8a and 8b exhibited most prominent and consistent anti-inflammatory activity with rapid onset of action and sustained duration till four hours. It is also obvious in compound 9b the substituted phenyl ring at 4-position in combination to sulfadiazine moiety at 7-position seems preferable the anti-inflammatory activity [31] with percentage inhibition of edema 67.85%. The data was represented in table 1.2.

Table 1: Anti-inflammatory evaluation of tested compounds

Group	% Change from baseline			
	1h	2hrs	3hrs	4hrs
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*
6a	46.16±3.685	40.29±2.566*	21.93±2.956*	24.00±3.265*
6b	90.80±1.62*	81.46±6.338	72.32±6.793*	56.31±3.259*
6c	46.30±1.852	56.32±2.822	51.28±3.454*	59.92±4.658*
7a	33.52±3.365	32.65±3.265*	44.25±6.632*	56.94±5.874*
7b	40.41±5.204	40.73±2.365*	45.71±5.810*	47.82±2.057*
7c	31.08±3.365	40.98±5.326*	54.37±6.235*	59.82±7.552*
8a	26.49±2.365*	50.33±6.233*	58.28±6.157*	65.35±6.338*
8b	28.55±2.369*	34.77±4.236*	45.36±5.236*	54.73±5.236*
9a	49.84±5.709	40.49±5.685*	38.38±2.412*	45.49±7.071*
9b	44.32±5.685	43.07±5.002*	38.26±7.737*	40.21±3.263*
10a	38.04±3.839	46.96±2.988*	55.85±2.246*	61.76±3.055*
10b	39.57±0.9936	43.67±2.796*	61.52±6.593*	71.17±7.386*
11a	42.76±2.356	46.15±5.263*	52.80±2.365*	56.66±2.365*
11b	47.08±4.898	48.31±5.251*	50.62±5335*	48.89±7.269*

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

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

Crosse	% Inhibition (Potency)			
Group	1h	2 hrs	3 hrs	4 hrs
Piroxicam	61.09	67.81	61.58	62.17
6a	35.61	61.7	81.67	80.71
6b	-29.65	22.56	39.37	54.98
6с	35.41	46.46	57.01	52.11
7a	53.24	68.96	62.9	54.48
7b	43.63	61.28	61.68	61.77
7c	56.64	61.04	54.42	52.18
8a	63.04	52.15	51.14	47.76
8b	60.17	66.9	61.97	56.25
9a	30.47	61.51	67.82	63.63
9b	38.17	59.05	67.921	67.85
10a	46.93	55.36	53.18	50.63
10b	44.8	58.48	48.18	43.10
<b>11</b> a	40.35	56.13	55.74	54.70
11b	34.32	54.07	57.56	60.91

### 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<sup>-1.</sup> In this method the

antinociceptive 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 **6c**, **8a**, **9a** and **10a**,**b** showed a significant analgesic activity compared to the untreated control group. The potency percentage of the analgesic activity of tested compounds ranged from 9.24 % with compound **11a** to 38.43 % with compound **10b** after 90 min., with no significant difference from piroxicam (39.72%). Aminopyrimidinthione ring which is known

to confer a sedative activity [32] seemed to enhance the analgesic activity of compounds **10a** and **10b**. Moreover, it is being found that compound **10b** possessed most rapid and sustained duration antinociceptive activity till 90 min. On the other hand, compounds **6a,b**, **7a,b**, **8b**, **9b** and **11b** showed moderate antinociceptive activity comparable to standard piroxicam positive control.

Groups	% Change from baseline		
	30 min	60 min	90 min
Control	$11.59 \pm 2.846$	$11.68 \pm 0.460$	6.966 ± 0.023
Piroxicam	$64.41 \pm 3.721$	$191.6 \pm 20.510*$	$283.6 \pm 12.236*$
ба	$18.20 \pm 5.391$	$57.45 \pm 5.119$	$90.17 \pm 5.368$
6b	$41.41 \pm 5.512$	$152.0 \pm 13.21$	$93.26 \pm 8.652$
6с	$38.55 \pm 2.612$	$110.3 \pm 1.236$	271.4 ± 20.213*
7a	$58.89 \pm 6.230$	$121.3 \pm 5.730$	$170.6 \pm 10.23$
7b	78.98 ±7.569	$63.62 \pm 8.562$	$83.19 \pm 6.987$
7c	82.18 ± 7.211	189.8 ± 6.604*	$136.8 \pm 7.365$
8a	86.92 ± 9.551	$133.3 \pm 6.985$	258.6 ± 20.315*
8b	$108.1 \pm 10.012*$	$59.57 \pm 2.763$	$135.8 \pm 5.689$
9a	$97.25 \pm 10.712$	$193.0 \pm 15.59^*$	237.7 ± 21.365*
9b	$61.47 \pm 8.252$	$96.54 \pm 8.965$	$158.1 \pm 13.265$
10a	$88.39 \pm 10.221$	$157.9 \pm 0.938$	231.1 ± 20.369*
10b	126.7 ± 11.236*	187.6 ± 15.12*	274.7 ± 9.12*
11a	55.81 ± 4.789	$127.5 \pm 11.18$	71.33 ± 8.796
11b	$98.08 \pm 5.265$	48.55 ± 3.340	113.9 ± 28.975

 Table 3: Analgesic evaluation of tested compounds

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

Table 4: %	Potency of	tested com	pounds after	30, 6	0 and 90 min
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Group	Potency from control		
	30 min	60 min	90 min
Piroxicam	45.570	15.404	39.712
ба	0.570	3.918	11.944
бb	2.572	12.013	12.384
6с	2.326	8.443	27.040*
7a	4.081	9.385	23.490
7b	5.814	4.446	11.049
7c	6.090	15.250	18.638
8a	6.499	10.412	36.123*
8b	8.327	4.100	18.494
9a	7.390	15.523	33.122*
9b	4.303	7.265	21.695
10a	6.626	12.518	32.175*
10b	9.931	15.061	38.434*
11a	3.815	9.916	9.239
11b	7.462	3.156	15.350

## **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, compound **10b** showed a significant antipyretic activity compared to the untreated control group. The potency percentage of the antipyrteic activity of tested compounds ranged from **35.68±0.096\*** with compound **9b** to **36.08±0.117\*** with compound **10b** after 2 hrs post yeast., with no significant difference from piroxicam **35.80±0.207**. Moreover, it is being found that compound 10b possessed excellent activity till 2 hrs post yeast. On the other hand, compounds **6a** and **9b** showed

antipyretic activity similar to standard piroxicam positive control. The data was represented in table 5.

<b>Fable 5: Antipyretic</b>	effects of the	tested com	pounds:
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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*
6a	35.68±0.1828	36.38±0.247	36.18±0.274	35.90±0.202*
9b	35.66±0.2015	36.20±0.141	35.96±0.107	35.68±0.096*
10b	35.66±0.2011	36.30±0.216	36.04±0.147	36.08±0.117*

The data represents the mean  $\pm$  standard error of the mean (n = 6).

Values represent the mean  $\pm$  S.E. of six animals for each groups.

P < 0.05: Statistically significant from Control. (One way Anova followed by Tuky 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. Table 6: Gastric ulcerative effect of test compounds compared to piroxicam

Group	Ulcer number	Ulcer severity
Control	-	-
Piroxicam	11.8±0.985*	22.4±1.652*
6a	-	-
6b	-	-
6с	-	-
7a	-	-
7b		
7c	-	-
8a	-	-
8b	-	-
9a	-	-
9b	-	-
10a	-	-
10b	-	-
<b>11</b> a	-	-
11b	-	-

Data is expressed as mean±SEM.

The data are the result of six rats/ experiment.

#### 4. Conclusion

The data revealed that, the investigation of antiinflammatory screening of all prepared compounds showed marked anti-inflammatory properties. Compound **6a** 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 antiinflammatory effect. On the other hand, compounds 6c, 8a, 9a and 10a,b recorded equipotent analgesic activity compared to standard drug. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds. Additionally, compound **10b** has excellent antipyretic

activity and compounds 6a and 9b have similar antipyretic activity compared to piroxicam activity after 2 hrs post yeast. 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.

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#### **References:**

- 1. Palomer, A.; Cabre, F.; Pascual, J.; Campos, J.; Trugillo, M. A.; Entrena, A.; Gallo, M. A.; Garcia, L.; Macleon, D and Espinosa, A., Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. J. Med. Chem. 2002, 45: 1402-1411.
- 2. Schmidt, K.J.: Büning, J.; Jankowiak, C and Fellermann, K., Crohn's Targeted Therapy: Myth or Real Goal?. Current Drug Discovery Technologies. 2009, 6: 290-298.
- 3. Li, C.S.; Brideau, C.; Chan, C.C.; Savoie, C.; Claveau, D.; Charleson, S.; Gordon, R.; Greig, G.; Gauthier, J.Y.; Lau, C.K.; Riendeau, D.; Thérien, M.; Wong, E and Prasit, P., Pyridazinones as Selective Cyclooxygenase-2 inhibitors. Bioorg. & Med. Chem. Lett. 2003, 13(4): 567-600.
- 4. Sui, Z.; Guan, J.; Ferro, M.P.; McCoy, K.; Wachter, M.P.; Murray, W.V.; Singer, M.; Steber, M.; Ritchie, D.M and Argentieri, D.C., 1,3-Diarylcycloalkano pyrazoles and diphenyl hydrazides as selective inhibitors of cyclooxygenase-2. Bioorg. & Med. Chem. Lett. 2000, 10(6): 601-604.
- 5. Balsamo, A.; Coletta, I.; Guglielmotti, A.; Landolfi, C.; Mancini, F.; Martinelli, A.; Milanese, C.; Minutolo, F.; Nencetti, S.; Orlandini, E.; Pinza, M.; Rapposelli, S and Rossello, A., Synthesis of heteroaromatic analogues of (2-aryl-1-cyclopentenyl-1alkylidene)-(arylmethyloxy)amine COX-2 inhibitors: effects on the inhibitory activity of the replacement of

the cyclopentene central core with pyrazole, thiophene or isoxazole ring. *Eur. J. Med. Chem.* 2003, *38*(2): 157-168.

- Khan, M.S.Y and Husain, A., Syntheses and reactions of some new 2-arylidene-4-(biphenyl-4-yl)-but-3-en-4olides with a study of their biological activity. *Pharmazie*. 2002, *57*(7): 448–452.
- Husain, A.; Khan, M.S.Y.; Hasan, S.M and Alam, M.M., 2-Arylidene-4-(4-phenoxy-phenyl)but-3-en-4olides:Synthesis, reactions and biological activity. *Eur. J. Med. Chem.* 2005, *40* (12): 1394–1404.
- Bhandari, S.V.; Bothara, K.G.; Raut, M.K.; Patil, A.A.; Sarkate, A.P and Mokale, V.J., Design, Synthesis and Evaluation of Antiinflammatory, Analgesicand Ulcerogenicity studies of Novel S-Substituted phenacyl-1,3,4-oxadiazole-2-thiol and Schiff bases of Diclofenac acid as Nonulcerogenic Derivatives. *Bioorg. & Med. Chem.* 2008, *16* (4): 1822–1831.
- Schoen, R.T and Vender, R.J., Mechanisms of nonsteroidal anti-inflammatory drug- induced gastric damage. Am. J. Med. 1989, 86: 449-458.
- Wolfe, M.M.; Lichtenstein, D.R and Singh, G., Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *Engl. J. Med.* 1999, *340:* 1889-1899.
- Kalgutkar, A.S.; Marnett, A.B.; Crews, B.C.; Remmel, R.P and Marnett, L.J., Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors. *J. Med. Chem.* 2000, *43*(150): 2860–2870.
- Murry, M.D and Brater, D.C., Renal toxicity of the nonsteroidal anti-inflammatory drugs. *Annu. Rev. Pharmacol. Toxicol.* 1993, 33: 435-465.
- Bombardier, C., An evidence-based evaluation of the gastrointestinal safety of Coxibs. *Review Am. J. Cardiol.* 2002, 89(6A): 3D-9D.
- 14. DeRuiter, J., Non-Steroidal Anti-inflammatory Drugs. Principles of Drug Action. 2002, 2: 1-25.
- Dogne, J.M.; Supuran, C.T and Pratico, D., Adverse cardiovascular effects of the coxibs. J. Med. Chem. 2005, 48: 2251-2257.
- 16. Bruno, O.; Schenone, S.; Ranise, A.; Bondavalli, F.; Filippelli, W.; Falcone, G.; Motola, G and Mazzeo, F., Antiinflammatory agents: new series of *N*substituted amino acids with complex pyrimidine structures endowed with antiphlogistic activity. *Il Farmaco*. 1999, *54*: 95–100.
- Zakia, M.A.; Solimana, H.A.; Hiekalb, O.A and Rashad, A.E., Pyrazolo pyrano pyrimidines as a Class of Anti-Inflammatory Agents. Z. Naturforsch. 2006, 61c: 1-5.
- Nofal, Z.M.; Fahmy, H.H.; Kamel, M.M.; Sarhan, A.I and Soliman, G.A., Synthesis of novel Pyrano[2,3c]pyrazoles and related fused ring system for the study of anti-inflammatory, analgesic and antipyretic activities. *Egypt J. Pharm. Sci.* 2003, *44*(2): 155-176.
- Winter, C.A.; Risley, E.A and Nuss, G.W., Carrageenin-induced oedema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 1962, *111*: 544-547.

- Mandour, A.H.; Eslam, R.; Manal, S.E and Seham, M.H., Synthesis and potential biological activity of some novel3-[(*N*-substitutedindol-3-yl)methyleneamino]-6amino-4-aryl-pyrano(2,3-c)pyrazole-5-carbonitriles and 3,6-diamino-4-(*N*-substituted indol-3-yl)pyrano(2,3c)pyrazole-5-carbonitriles. *Acta Pharm.* 2012, 62: 15-30.
- Chattopadhyay, D.; Arunachalam, G.; Mandal, A.B.; Sur, T.K.; Mandal, S.C and Bhattacharya, S.K., Antimicrobial and anti-inflammatory activity of folklore: Mallotus peltatus leaf extract. *J. Ethnopharmacol.* 2002, 82: 229-237.
- 22. Nishiyama, T.; Gyermek, L.; Lee, C.; Kawasaki-Yatsugi, S and Yamaguchi, T., The Systemically Administered Competitive AMPA Receptor Antagonist, YM872, has Analgesic Effects on Thermal or Formalin-Induced Pain in Rats. *Anesth Analg.* 1999, *89(6):* 1534–1547.
- 23. Szelenyi, I and Thiemer, K., Distention ulcer as a model for testing of drugs for ulcerogenic side effects. *Archives of Toxicology*. 1978, *41:* 99-105.
- 24. Santos, L.H.; Feres, C.A.O.; Melo, F.H.; Coelho, M.M.; Nothenberg, M.S.; Oga, S and Tagliati, C.A., Anti-inflammatory, antinociceptive and ulcerogenic activity of a zinc-diclofenac complex in rats. *Braz. J. Med. Biol. Res.* 2004, *37*(8): 1205-1213.
- 25. Sturz, H.G and Noller, C.R., Some substituted benzylidenemalononitriles. J. Am. Chem. Soc. 1949, 71: 2949-2956.
- Taher, A.T.; Georgey, H.H and El-Subbagh, H.I., Novel 1,3,4-heterodiazole Analogues: Synthesis and invitro antitumor activity. *Eur. J. Med. Chem.* 2012, 47: 445-451.
- Elkholy, Y.M and Morsy, M.A., Facile Synthesis of 5,6,7,8-Tetrahydropyrimido [4,5-b]-quinoline Derivatives. *Molecules*. 2006, *11*: 890-903.
- Lin, H.C.; Tsai, S.H.; Chen, C.S.; Chang, Y.C.; Lee, C.M.; Lai, Z.Y and Chun-Mao Lin, C.M., Review on Synthesis and Various Biological Potential of Thiazolo pyrimidine Derivatives. *Biochem. Pharm.* 2008, 75: 1416-1425.
- Redda, K.K.; Rao, K.N.; Heiman, A.S.; Onayemi, F.Y and Clark, J.B., Synthesis and Anti-inflammatory activities of some N-[pyridyl(phenyl)carbonylamino]tert-butyl/ phenyl-1,2,3,6- tetrahydropyridines. *Chem. Pharm. Bull. Tokyo.* 1991, *39*(3): 786-791.
- 30. Ruiz, J.; López, M.; Milà, J.; Lozoya, E.; Lozano, J.J and Pouplana, R., QSAR and Conformational Analysis of the Anti-inflammatory agent Amfenac and Analogues. J. Comput Aided Mol Des. 1993, 7(2): 183-198.
- Husain, A.; Ahmad, A.; Mujeeb, M and Akhter, M., New Amides of Sulphonamides: Synthesis and Biological Evaluation. J. Chil. Chem. Soc. 2010, 55(1): 74-77.
- 32. Kashyap, S.J.; Sharma, P.K.; Garg, V.K.; Dudhe, R and Kumar, N., Synthesis and Various Biological Potentials of Thiazolo pyrimidine Derivatives. *J. Adv. Sci*.*Res.* 2011, *2*(*3*): 18-24.