

Synthesis and pharmacological evaluation of pyrazolopyrimidopyrimidine derivatives: anti-inflammatory agents with gastroprotective effect in rats

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Abstract We report the synthesis of new anti-inflammatory 1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine **5** from aminocyanopyrazole. All compounds were characterized by physical, chemical and spectral studies. Preliminary pharmacological evaluation of the resulting products showed that compounds **5a**, **b**, **f** (50–100 mg/kg, i.p.) are active anti-inflammatory agents in carrageenan-induced rat paw oedema assay, and their effects are comparable to that of acetylsalicylic–lysine (300 mg/kg, i.p.), used as a reference drug. The nature of substituent (Y, R₃) had a pronounced effect on the anti-inflammatory activity. Studies of structure–activity relationships have led to selection of compound ethyl-3,5-dimethyl-7-imino-*N*¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine-6-carboxylate, **5f** which exhibited the most potent anti-inflammatory activity. In addition, the compounds **5a**, **b**, **f** showed a significant gastroprotective effect against HCl/EtOH-induced gastric ulcer.

Keywords Aminocyanopyrazole · Anti-inflammatory · Gastroprotective · Pyrazolo[3,4-*d*]pyrimidine · Dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are most widely used to treat variety of acute and chronic inflammatory diseases. Such drugs are being increasingly used for the treatment of postoperative pain (Moote, 1992) with or without supplemental opioid agents. The pharmacological action of these agents was assigned to inhibit two enzymes, known as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Vane *et al.*, 1998). The constitutive isoform COX-1 is present in most tissues and is involved in the synthesis of prostaglandins vital to normal cell function. In contrast, the inducible isoform COX-2 appears to be produced primarily in response to growth factors or inflammatory mediators, such as cytokines (Vane and Botting, 1996). Many of the currently available NSAIDs, including indomethacin and piroxicam, are more potent inhibitors of COX-1 than that of COX-2 (Vane and Botting, 1995). This preferential inhibition of COX-1 may be responsible for many of the adverse effects associated with NSAIDs. It has been postulated that NSAIDs which preferentially inhibit COX-2, such as meloxicam (Lipscomb *et al.*, 1998), celecoxib (Simon *et al.*, 1998) and several experimental drugs including NS 398, L-745,337 and DFP, should produce the same or better anti inflammatory effects with less gastrointestinal, haematological and renal toxicities than classical NSAIDs (Winter *et al.*, 1962). Pyrazolopyrimidines are a class of sedative and anxiolytic drugs such as Zaleplon known by its hypnotic effect (Weitzel *et al.*, 2000). However, pyrazolopyrimidine derivatives become a new chemical resource for searching of novel bioactive compounds in drug development.

On this basis, we directed our attention to the synthesis of novel 1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidines **5a–i** related to aminocyanopyrazole with the

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aim of improving their anti-inflammatory activity and reducing their ulcerogenic properties as it appeared to be plausible that variation of the active compound structures could exert a pronounced influence on activity, as the case with **5b**, **f**.

Materials and methods

Chemistry

Phenyl hydrazine, malononitrile, triethylorthoester and ammoniac were purchased from Sigma Chemical (Berlin, Germany). Analytical grade solvents (ethanol, HCl, ethyl acetate, chloroform) were obtained from Merck.

Melting points (mp) were determined on a Buchi capillary apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 300 spectrometer (^1H at 300 MHz and ^{13}C at 75 MHz) with deuterio-dimethylsulphoxide (*d*-DMSO) as solvent and tetramethylsilane as internal standard reference. Infra-red (IR) spectra were recorded on a Bio-rad FTS-6000 spectrometer. Solvents used in reactions were dried and distilled before use. The purity of all synthesized compounds was controlled by thin layer chromatography (TLC; Merck silica gel plates 60F-254). High resolution masses were recorded on a spectrometer JEOL JMS-Gcmate II is composed of a GC/MS system from compounds dissolved in dichloromethane.

Synthesis and spectral data of compounds 2–5

5-Amino-4-cyano-*N*¹-phenyl pyrazoles (2) 5-Amino-4-cyano-1-*N*¹-phenyl pyrazoles prepared via a standard addition of hydrazine derivatives to ketene ethoxymethylene compounds following the reported procedure. Recrystallization from ethanol afforded pure **2** in good yields.

4-Cyano-*N*¹-phenyl pyrazolo-5-imidates (3) The required 5-amino-4-cyano-*N*¹-phenyl pyrazole (1.0 mmol) was treated with triethylorthoester (6.0 mmol) and a catalytic amount of acetic acid and the mixture was refluxed for 24 h. After cooling, the reaction mixture was evaporated. The product was filtered, washed with diethyl ether then purified by recrystallisation (ethanol) (Gupta *et al.*, 2008; Allouche *et al.*, 2013).

4-Amino-*N*¹-phenyl pyrazolo[3,4-*d*]pyrimidine (4) A solution of imidate **3** (1.0 mmol) in dry ethanol (5 ml) was treated with ammoniac (2.0 mmol) and a catalytic amount of acetic acid. The reaction mixture was refluxed for 6 h, and the formed solid was collected by filtration, dried and recrystallized from ethanol to give compound **4**.

- a) **4-Amino-*N*¹-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 4a** Yield 83 %; mp 228 °C; IR (cm^{-1}); ν_{NH_2} 3100, 3283; $\nu_{\text{C}=\text{N}}$ 1480, 1500, 1590 cm^{-1} ; RMN ^1H (δ ppm, DMSO): 4.69 (2H, s, NH_2), 7.36 (1H, t, $J = 7.3$ Hz, ArH_4), 7.48 (2H, t, $J = 7.3$ Hz, ArH_3 and ArH_5), 7.52 (2H, d, $J = 7.3$ Hz, ArH_2 and ArH_6), 7.60 (1H, s, H_3), 7.72 (1H, s, H_6), ^{13}C RMN (δ ppm, DMSO): 114.14 (C-3a), 124.27 (C-2' and C-6'), 129.00 (C-4'), 129.58 (C-3' and C-5'), 130.04 (C-3), 136.94 (C-1'), 141.36 (C-7a), 149.83 (C-6), 156.84 (C-4); HRMS Calcd. for $\text{C}_{11}\text{H}_9\text{N}_5$: 211.0858, found: 211.0859.
- b) **4-Amino-3-methyl-*N*¹-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 4b** Yield 68 %; mp 192 °C; IR (cm^{-1}); ν_{NH_2} 3083, 3317; $\nu_{\text{C}=\text{N}}$ 1626, 1647, 1665; RMN ^1H (δ ppm, DMSO): 2.76 (3H, s, CH_3), 5.97 (2H, s, NH_2), 7.33 (1H, t, $J = 7.1$ Hz, ArH_4), 7.57 (2H, t, $J = 7.1$ Hz, ArH_3 and ArH_5), 8.16 (2H, d, $J = 7.1$ Hz, ArH_2 and ArH_6), 8.46 (1H, s, H_3); RMN ^{13}C (δ ppm, DMSO): 14.89 (CH_3), 101.23 (C-3a), 121.49 (C-2' and C-6'), 126.37 (C-4'), 129.19 (C-3' and C-5'), 138.81 (C-3), 141.83 (C-1'), 154.41 (C-7a), 156.48 (C-4), 158.40 (C-6); HRMS Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_5$: 225.1014, found: 225.1018.
- c) **4-Amino-6-methyl-*N*¹-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 4c** Yield 70 %; mp 160 °C; IR (cm^{-1}); ν_{NH_2} 3090, 3320; $\nu_{\text{C}=\text{N}}$ 1597, 1638, 1663; RMN ^1H (δ ppm, DMSO): 2.65 (3H, s, CH_3), 4.28 (2H, s, NH_2), 7.28 (1H, t, $J = 7.3$ Hz, ArH_4), 7.56 (2H, t, $J = 7.3$ Hz, ArH_3 and ArH_5), 8.19 (2H, d, $J = 7.3$ Hz, ArH_2 and ArH_6), 8.29 (1H, s, H_6); RMN ^{13}C (δ ppm, DMSO): 14.44 (CH_3), 100.24 (C-3a), C_{arom} 120.24 (C-2' and C-6'), 124.67 (C-4'), 129.16 (C-3' and C-5'), 138.8 (C-3), 142.79 (C-1'); C_3 154.14 (C-7a), 156.51 (C-4), 158.58 (C-6); HRMS Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_5$: 225.1014, found: 225.1016.

7-Imino-*N*¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine 5a–e A mixture of compound **4** (1.0 mmol), ketene ethoxymethylene compounds **1** or ethyl-2-cyano-3-ethoxyalkyl-2-enoate (1.0 mmol) and a catalytic amount of acetic acid was refluxed for 2 h in 10 ml ethanol. The formed precipitate was filtered, washed by diethyl ether, dried and recrystallized from ethanol to give compound **5** in good yield.

- a) **6-Cyano-7-imino-3-methyl-*N*¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine 5a** Yield 68 %; mp 290 °C; IR (cm^{-1}); ν_{NH} 3356; $\nu_{\text{C}=\text{N}}$ 2212; $\nu_{\text{C}=\text{N}}$ 1534, 1554, 1587; RMN ^1H (δ ppm, DMSO): 2.51 (3H, s, CH_3); 7.38 (1H, t, $J = 7.3$ Hz, ArH_4); 7.53 (2H, t, $J = 7.3$ Hz, ArH_3 and ArH_5); 7.71 (2H, d, $J = 7.3$ Hz, ArH_2 and ArH_6); 8.02 (1H, s, H_5);

- 8.38 (1H, s, H₉); 8.66 (1H, s, NH); RMN¹³C (δ ppm, DMSO): 14.64 (CH₃); 91.81 (C-6); 105.88 (C-3a); 116.24 (CN); C_{arom} 120.46 (C-2' and C-6'), 124.17 (C-4'), 129.27 (C-3' and C-5'), 137.89 (C-1'), 143.42 (C-10a), 149.71 (C-3), 159.61 (C-5), 161.88 (C-9), 162.15 (C-4a); 163.43 (C-7); HRMS Calcd. for C₁₆H₁₁N₇: 301.1076, found: 301.1051.
- b) *6-Cyano-7-imino-3,5-dimethyl-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine 5b* Yield 54 %; mp 182 °C; IR (cm⁻¹): ν_{NH} 3324; ν_{C≡N} 2230; ν_{C=N} 1509, 1562, 1586; RMN¹H (δ ppm, DMSO): 2.50 (3H, s, CH₃), 2.64 (3H, s, CH₃); 7.26 (1H, t, *J* = 7.3 Hz, ArH₄); 7.51 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 7.54 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 8.19 (1H, s, H₉); 8.27 (1H, s, NH); RMN¹³C (δ ppm, DMSO): 14.42 (CH₃); 21.00 (CH₃); 87.23 (C-6); 100.25 (C-3a); 109.00 (CN); 120.22 (C-2' and C-6'), 125.51 (C-4'), 128.98 (C-3' and C-5'), 138.89 (C-1'); 142.79 (C-10a); 154.17 (C-3), 156.49 (C-5), 164.59 (C-9), 165.71 (C-4a), 167.94 (C-7); HRMS Calcd. for C₁₇H₁₃N₇: 315.1232, found: 315.1214.
- c) *6-Cyano-7-imino-9-methyl-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine 5c* Yield 71 %; mp 166 °C; IR (cm⁻¹): ν_{NH} 3321.86; ν_{C≡N} 2223, 1536, 1561, 1599; RMN¹H (δ ppm, DMSO): 2.62 (3H, s, CH₃); 7.40 (1H, t, *J* = 7.3 Hz, ArH₄); 7.49 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 7.68 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 8.19 (1H, s, H₅); 8.41 (1H, s, H₉); 8.73 (1H, s, NH); RMN¹³C (δ ppm, DMSO): 14.32 (CH₃); 89.64 (C-6); 103.64 (C-3a); 111.83 (CN); C_{arom} 120.38 (C-2' and C-6'), 126.65 (C-4'), 138.42 (C-3' and C-5'), 140.12 (C-1'), 143.42 (C-10a), 141.69 (C-3), 148.47 (C-5), 160.28 (C-9), 161.92 (C-4a); 162.00 (C-7). C₁₆H₁₁N₇: 301.1051; HRMS Calcd. for C₁₆H₁₁N₇: 301.1076, found: 301.1087.
- d) *6-Cyano-7-imino-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine 5d* Yield 77 %; mp 248 °C; IR (cm⁻¹): ν_{NH} 3189; ν_{C≡N} 2250; ν_{C=N} 1532, 1559, 1562; RMN¹H (δ ppm, DMSO): 7.33 (1H, t, *J* = 7.3 Hz, ArH₄), 7.55 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅), 8.03 (1H, s, H₅), 8.21 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆), 8.31 (1H, s, H₉), 8.36 (1H, s, H₃), 8.37 (1H, s, NH); RMN¹³C (δ ppm, DMSO): 89.87 (C-6); 101.37 (C-3a); 120.45 (CN); C_{arom} 126.00 (C-2' and C-6'), 129.10 (C-4'), 130.15 (C-3' and C-5'), 134.04 (C-1'); 138.94 (C-10a); 139.11 (C-3); 142.14 (C-5); 153.19 (C-9); 156.68 (C-4a); 158.26 (C-7); HRMS Calcd. for C₁₅H₉N₇: 287.0976, found: 287.0919.
- e) *6-Cyano-7-imino-5-ethyl-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine 5e* Yield 70 %; mp 168 °C; IR (cm⁻¹): ν_{NH} 3332; ν_{C≡N} 2218; ν_{C=N} 1568, 1589, 1620; RMN¹H (δ ppm, DMSO): 1.23 (3H, t, CH₃); 2.30 (2H, q, CH₂); 7.30 (1H, t, *J* = 7.3 Hz, ArH₄); 7.52 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 8.04 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 8.18 (1H, s, H₅); 8.52 (1H, s, H₉); 11.16 (1H, s, NH); RMN¹³C (δ ppm, DMSO): 9.01 (CH₃); 29.31 (CH₂); 92.54 (C-6); 106.31 (C-3a); 114.07 (CN); C_{arom} 121.28 (C-2' and C-6'), 124.73 (C-4'), 126.56 (C-3' and C-5'), 141.13 (C-1'), 145.82 (C-10a), 152.63 (C-3), 155.28 (C-9), 161.23 (C-4a), 162.07 (C-7); 165.49 (C-5); HRMS Calcd. for C₁₇H₁₃N₇: 315.1232, found: 315.1352.
- f) *Ethyl-3,5-dimethyl-7-imino-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine-6-carboxylate 5f* Yield 71 %; mp 170 °C; IR (cm⁻¹): ν_{NH} 3081; ν_{CO} 1747; ν_{C=N} 1510, 1565, 1590; RMN¹H (δ ppm, DMSO) 1.21 (3H, t, *J* = 7.2 Hz, CH₃); 1.91 (3H, s, CH₃); 2.62 (3H, s, CH₃); 4.15 (2H, q, *J* = 7.2 Hz, CH₂); 7.28 (1H, t, *J* = 7.3 Hz, ArH₄); 7.51 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 8.17 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 8.26 (1H, s, H₉); 11.97 (1H, s, NH). RMN¹³C (δ ppm, DMSO) 13.01 (CH₃); 14.00 (CH₃); 24.45 (CH₃); 66.03 (CH₂); 105.28 (C-6); 115.10 (C-3a); 121.07 (C-2' and C-6'), 125.50 (C-4'), 129.12 (C-3' and C-5'), 138.88 (C-1'), 142.79 (C-10a), 146.88 (C-3), 148.30 (C-5), 154.14 (C-9), 156.21 (C-4a), 156.48 (C-7), 164.27 (CO); HRMS Calcd. for C₁₉H₁₈N₆O₂: 362.1491, found: 362.1478.
- g) *Ethyl-5-ethyl-7-imino-3-methyl-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine-6-carboxylate 5g* Yield 69 %; mp 181 °C; IR (cm⁻¹): ν_{NH} 3081; ν_{CO} 1706; ν_{C=N} 1434, 1493, 1589; RMN¹H (δ ppm, DMSO) 1.06 (3H, t, *J* = 7.1 Hz, CH₃); 1.34 (3H, t, *J* = 7.0 Hz, CH₃); 1.97 (2H, q, *J* = 7.1 Hz, CH₂); 2.63 (3H, s, CH₃); 4.03 (2H, q, *J* = 7.0 Hz, CH₂); 7.49 (1H, t, *J* = 7.3 Hz, ArH₄); 7.63 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 8.03 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 9.57 (1H, s, H₉); 11.96 (1H, s, NH). RMN¹³C (δ ppm, DMSO) 11.26 (CH₃); 14.03 (CH₃); 14.07 (CH₃); 30.19 (CH₂); 67.92 (CH₂); 105.58 (C-6); 114.96 (C-3a); 120.64 (C-2' and C-6'), 125.99 (C-4'), 129.69 (C-3' and C-5'), 139.45 (C-1'), 143.25 (C-10a), 154.76 (C-3), 156.97 (C-5), 159.15 (C-9), 162.04 (C-4a), 162.50 (C-7), 164.09 (CO); HRMS Calcd. for C₂₀H₂₀N₆O₂: 376.1648, found 376.1621.
- h) *Ethyl-7-imino-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine carboxylate 5h* Yield 89 %; mp 184 °C; IR (cm⁻¹): ν_{NH} 3227; ν_{CO} 1710; ν_{C=N} 1539, 1552, 1574.17; RMN¹H (δ ppm, DMSO) 1.29 (3H, t, *J* = 7.0 Hz, CH₃); 4.24 (2H, q, *J* = 7.0 Hz, CH₂); 7.37 (1H, t, *J* = 7.3 Hz, ArH₄); 7.55 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 8.14 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 8.75 (1H, s, H₅); 8.83 (1H, s, H₉); 9.18 (1H, s, H₃); 12.11 (1H, s, NH). RMN¹³C (δ ppm, DMSO) 14.11 (CH₃); 61.36 (CH₂); 103.83 (C-6); 114.46 (C-3a); 120.62 (C-2' and C-6'), 126.73 (C-4'), 129.20 (C-3'

and C-5'), 134.35 (C-1'), 138.10 (C-10a), 148.14 (C-3), 151.37 (C-5), 153.53 (C-9), 154.00 (C-4a), 155.18 (C-7), 163.36 (CO). 120.62–126.73–129.20–134.35, C₁₇H₁₄N₆O₂, 334.1171; HRMS Calcd. for: C₁₇H₁₄N₆O₂: 334.1178, found: 334.1171.

- i) *Ethyl-5-methyl-7-imino-N¹-phenyl-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-a]pyrimidine-6-carboxylate 5i* Yield 78 %; mp 166 °C; IR (cm⁻¹); ν_{NH} 3059; ν_{CO} 1718; ν_{C=N} 1579, 1591, 1612; RMN ¹H (δ ppm, DMSO) 1.34 (3H, t, *J* = 7.0 Hz, CH₃); 1.92 (3H, s, *J* = 7.1 Hz, CH₃); 4.02 (2H, q, *J* = 7.0 Hz, CH₂); 7.30 (1H, t, *J* = 7.3 Hz, ArH₄); 7.61 (2H, t, *J* = 7.3 Hz, ArH₃ and ArH₅); 8.10 (2H, d, *J* = 7.3 Hz, ArH₂ and ArH₆); 9.29 (1H, s, H₃); 9.49 (1H, s, H₉); 11.95 (1H, s, NH). RMN¹³C (δ ppm, DMSO); 15.06 (CH₃); 23.14 (CH₃); 69.54 (CH₂); 102.85 (C-3a); 117.05 (C-6); 121.637 (C-2' and C-6'), 126.41 (C-4'), 128.65 (C-3' and C-5'), 139.24 (C-1'), 143.92 (C-10a), 144.17 (C-3), 159.62 (C-5), 161.45 (C-9), 167.12 (C-4a), 167.83 (C-7), 168.28 (CO); HRMS Calcd. for C₁₈H₁₆N₆O₂: 348,1335, found 348,1274.

Pharmacology

Carrageenan (BDH Chemicals Ltd., Poole, England), cimetidine and acetylsalicylic–lysine were purchased from pharmacie Centrale of Tunisia.

Animals

Adult Male Wistar rats weighing 150–170 g were obtained from Pasteur Institute (Tunis, Tunisia). They were housed in polypropylene cages and left for 2 days for acclimatization to animal room maintained under controlled conditions: a 12 h light–dark cycle (at 22 ± 2 °C) on standard pellet diet and water ad libitum. Rats were fasted overnight with free access to water before the experiments. Housing conditions and in vivo experiments were approved, according to the guidelines established by the European Union on Animal Care (Communautés Économiques Européennes Council [86/609]).

Carrageenan-induced rat paw oedema

The anti-inflammatory activity of compounds (**5a**, **b**, **f**, **g**) on carrageenan-induced rat paw oedema was determined according to Winter *et al.* (1962). The animals were divided into three groups of six rats each. The control group received intraperitoneally 2.5 ml/kg of vehicle solution (Tween 80/absolute ethanol/saline solution (0.9 %) in the ratio 1:1:18). The reference group received acetylsalicylic–lysine (300 mg/kg i.p.), and the test groups received compounds **5a**, **b**, **f**, **g** (50 and 100 mg/kg, i.p.). After

30 min, 0.05 ml of 1 % carrageenan suspension was injected into the left hind paw. The paw volume up to the tibiotarsal articulation was measured using a plethysmometer (model 7150, UgoBasile, Italy) at 0 h (*V*₀) (before carrageenan injection) and 1, 3 and 5 h later (*V*_T) (after carrageenan injection). Paw swelling was determined for each rat and the difference between *V*_T and *V*₀ was taken as the oedema value. The percent inhibition was calculated according to the following formula:

$$\% \text{ Inhibition} = \left[\frac{(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated}}}{(V_T - V_0)_{\text{control}}} \right] \times 100$$

Gastroprotective activity

The gastroprotective activity of pyrazolopyrimidopyrimidines **5a**, **b**, **f**, **g** was studied in 150 mM HCl/EtOH-induced gastric ulcer (Hara and Okabe, 1985). Rats were fasted for 24 h prior receiving any treatment and were divided into six groups of six animals each. Group I was kept as control group and received the vehicle (Tween 80/Absolute ethanol/Saline solution (0.9 %): 1/1/18). Group II and III received compound **5a** (50, 100 mg/kg, i.p.), respectively, and Group IV and V received compound **5b** (50, 100 mg/kg, i.p.), respectively. Group VI and VII received compound **5f** (50, 100 mg/kg, i.p.), respectively, and group VIII and IX received compound **5g** (50, 100 mg/kg, i.p.), respectively. Group X received cimetidine (100 mg/kg, i.p.) as reference drug. After 30 min, all groups were orally treated with 1 ml/100 g of 150 mM HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. Animals were sacrificed 1 h after the administration of ulcerogenic agent; their stomachs were excised and opened along the great curvature, washed and stretched on cork plates. The surface was examined for the presence of lesions and the extent of the lesions was measured. The summative length of the lesions along the stomach was recorded (mm) as lesion index.

Statistics

Results are expressed as the mean ± SEM of six animals per group. The data were analysed using Student's *t* test, **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 was considered significant.

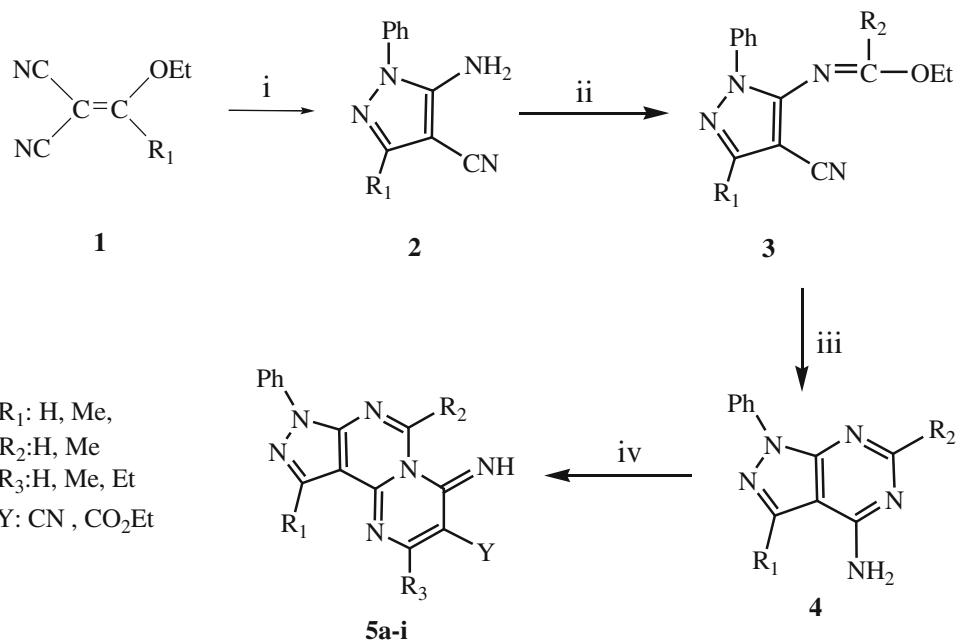
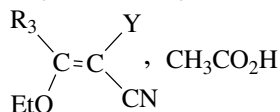
Results and discussion

Chemistry

The synthetic routes to target compounds **5a–i** are outlined in Scheme 1. The 5-amino-4-cyano-*N*¹-phenylpyrazole **2**,

Scheme 1 Synthetic procedure

of compounds **5a–i**. Reagents:
i H₂N–NHPH, CH₃CO₂H,
 CH₃CO₂H; *ii* R₂C(OEt)₃,
 CH₃CO₂H; *iii* NH₃; *iv*



used as a starting material, was prepared in two steps following a similar method reported by Petrie *et al.* (1985), Anderson *et al.*, (1990), Aggarwal *et al.*, (2011). The first step involves acid-catalysed condensation of orthoester with malonate to form ethoxymethylene malononitrile **1**. This later reacts then with substituted hydrazine to give the aminocyanopyrazole **2**. Treatment of **2** with orthoester in the presence of catalytic amount of acid furnished the corresponding cyano-pyrazoloimidates **3** which subsequently were transformed to the corresponding amino pyrazolopyrimidines **4** (Booth *et al.*, 1999; Gupta *et al.*, 2008; Oliveira-Campos *et al.*, 2007; Bakavoli *et al.*, 2010) upon treatment with ammoniac. Reaction of compound **4** with ketene ethoxymethylene compounds **1** in ethanol in presence of catalytic amount of acid furnished the desired 6-cyano-1,7-dihydropyrazolo[3',4':4,5]pyrimido[1,6-*a*]pyrimidine **5a–e** in 70 % yield as a yellow solid. The same procedure gave a crystalline ethyl-1,7-dihydro pyrazolo [3',4':4,5]pyrimido [1,6-*a*]pyrimidine-6-carboxylate **5f–i** from ethyl-2-cyano-3-

ethoxyalkyl-2-enoate in 80 % yield. Scheme 1 shows the synthetic strategy to obtain the target compounds by the four-steps method, yielding the compounds with structure **5a–i** listed in Table 1.

It is interesting to note that time reaction and yield of products are directly related to the nature of substituent (R₃ and Y). The yields of compounds **5h** and **5d** are 89 and 77 %, respectively. Hydrogen substituent R³ gave superior yields in short time. In all cases, reaction leads to pyrazolo pyrimido pyrimidine only when R¹ or R² is a hydrogen atom. However, steric effect decreased yields of the reaction, as in the case of **5g**, and may even prevent the progress of the reaction when R² and R³ are methyl groups. Analysis of the NMR and IR spectra indicated that compounds **5f–i** has ester functional group in their structures so ethoxymethylene cyanoacetate reacts with pyrazolopyrimidine and in both cases Y is CN or CO₂Et, nitrogen attacked on the nitrile function as the first attack.

Table 1 Synthesis of 7-imino-*N*¹-phenyl-1,7-dihydro pyrazolo[3',4':4,5]pyrimido [1,6-*a*]pyrimidine **5a–i**

Compounds	R ₁	R ₂	R ₃	Y	Yields (%)	Reaction time (h)
5a	CH ₃	H	H	CN	68	24
5b	CH ₃	H	CH ₃	CN	54	71
5c	H	CH ₃	H	CN	71	24
5d	H	H	H	CN	77	5
5e	H	H	C ₂ H ₅	CN	70	48
5f	CH ₃	H	CH ₃	CO ₂ Et	71	75
5g	CH ₃	H	C ₂ H ₅	CO ₂ Et	69	84
5h	H	H	H	CO ₂ Et	89	7
5i	H	H	CH ₃	CO ₂ Et	78	24

Fig. 1 Anti-inflammatory effect of the intraperitoneal administration of **5a**, **b**, **f**, **g** and of the reference drug (acetylsalicylic–lysine: ASL) in carrageenan-induced rat paw oedema. The values represent the means difference of volume of paw \pm SEM ($n = 6$). * $p < 0.01$ and ** $p < 0.001$ significantly different from the control group

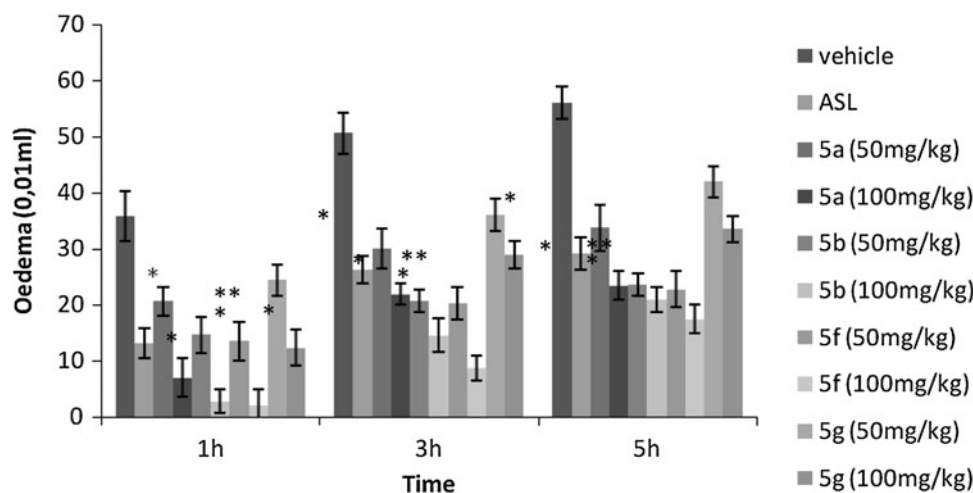
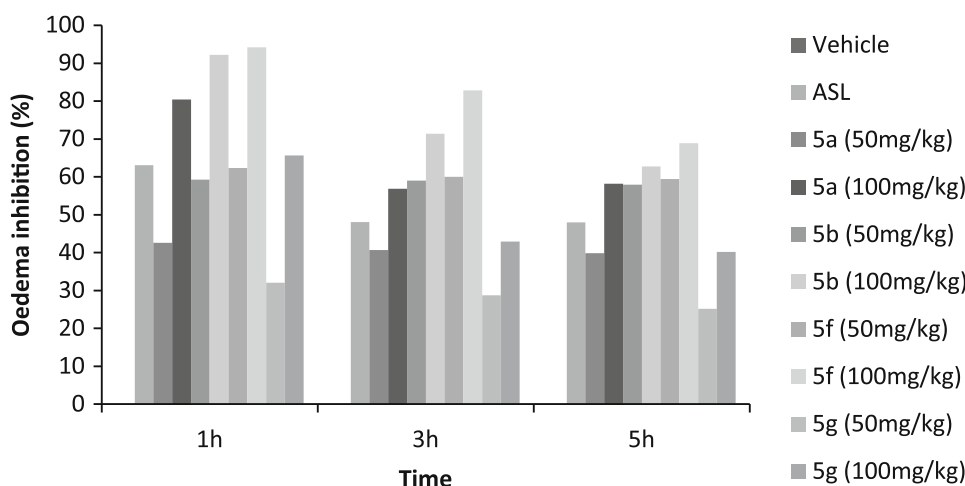


Fig. 2 Percentage inhibition of the oedema after the intraperitoneal administration of **5a**, **b**, **f**, **g** and the reference drug (acetylsalicylic–lysine: ASL) in carrageenan-induced rat paw oedema



Biological activity

Anti-inflammatory and gastroprotective activities of compounds **5a**, **b**, **f**, **g**

The pyrazolopyrimidine derivatives are a well-known class of NSAIDs with several products in market (Russo *et al.*, 1992; El-Kateb *et al.*, 2012) (Figs. 1, 2).

The structure–activity relationships (SAR) for these compounds have been extensively explored for optimization of anti-inflammatory activity last three decades, since this class was introduced (Lombardino and Wiseman, 1972; Farré *et al.*, 1986; Berq *et al.*, 1999; Lee *et al.*, 1999). In continual efforts to find potentially safer and more efficacious parent agents through further exploration of SAR of this class, we decided to study the pharmacological profiles of compounds **5a**, **b**, **f**, **g** belonging to pyrazolopyrimidopyrimidine family. We examined the effect of modification of the electronic nature of substituents on various portions of type NSAIDs. For this objective the hydrogen atom (position 5) is replaced by methyl or ethyl

group, even and for more important anti-inflammatory activity, the cyano function is replaced by ester function.

Table 2 reveals the results of the intraperitoneal administration of the compounds **5a**, **b**, **f**, **g** in carrageenan-induced rat paw oedema. The compounds **5a**, **b**, **f**, **g** tested at 50 and 100 mg/kg, i.p. produced a significant reduction of the oedema throughout the entire period of observation in a dose-dependent manner. The highest reduction of the oedema was at 3 h after carrageen injection with a percent inhibition ranged, from 40.64 to 56.81 % for compound **5a**, from 58.98 to 71.36 % for compound **5b**, from 60.02 to 82.83 % for compound **5f** and from 28.75 to 42.87 % for compound **5g**, whereas the reference drug (acetylsalicylic–lysine, 300 mg/kg, i.p.) produced 48.03 % reduction in paw volume. The influence of the substituent R_2 on activity is remarkable. Compound **5a** is less potent than the 5-methyl derivatives **5b**, so a methyl group linked to the pyrimidine cycle increases the activity compared to the case of a hydrogen atom. At the same dose (100 mg/kg), compound **5b** produced 71.36 % inhibition of oedema against 56.81 % for **5a**. In addition, the compound **5f** is

Table 2 Anti-inflammatory effect of the intraperitoneal administration of **5a**, **b**, **f**, **g** and of the reference drug (acetylsalicylic–lysine: ASL) in carrageenan-induced rat paw oedema

Sample	Dose (mg/kg)	Oedema (10 ⁻² ml) (mean ± SEM)			Oedema inhibition (%)		
		1 h	3 h	5 h	1 h	3 h	5 h
Vehicle (2.5 ml/kg)	–	35.87 ± 4.48	50.66 ± 3.68	56.04 ± 2.91	–	–	–
Acetylsalicylic–lysine (reference drug)	300	13.23 ± 2.69**	26.32 ± 2.44**	29.15 ± 2.87**	63.10	48.03	47.98
5a	50	20.59 ± 2.51*	30.07 ± 3.51*	33.73 ± 4.16*	42.59	40.64	39.8
	100	7.01 ± 3.41**	21.88 ± 1.89**	23.45 ± 2.5**	80.44	56.81	58.15
5b	50	14.62 ± 3.21*	20.78 ± 2*	23.56 ± 2*	59.25	58.98	57.95
	100	2.81 ± 2.06***	14.51 ± 2.98***	20.86 ± 2.21***	92.17	71.36	62.76
5f	50	13.51 ± 3.4**	20.25 ± 2.8**	22.74 ± 3.2**	62.31	60.02	59.42
	100	2.07 ± 2.8***	8.69 ± 2.3***	17.45 ± 2.5***	94.22	82.83	68.85
5g	50	24.37 ± 2.7*	36.09 ± 2.9*	41.95 ± 2.8	32.04	28.75	25.13
	100	12.31 ± 3.2**	28.94 ± 2.4*	33.52 ± 2.3	65.66	42.87	40.18

The values represent the mean difference of volume of paw ± SEM ($n = 6$)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from control group

more potent than the ethyl derivatives **5g**, so an ethyl group linked to the pyrimidine cycle decreases the activity compared to the methyl group.

On the other hand, mucosal erosion and ulceration are produced by most NSAIDs with varying degrees. Inhibition of synthesis of gastroprotective prostaglandins (PGE₂) is clearly involved (Nezamis *et al.*, 1967) and due to the inhibition of the constitutive isoform COX-1 (Main and Whittle, 1973; Cryer and Feldman, 1992). Thus, deficiency of PGs reduces the mucosal secretions along with hydrogen carbonate that ultimately aggravates the lethal effects of

acid on the stomach lining leading to mucosal damage (Fig. 3).

The results of gastroprotective activity of compounds **5a**, **b**, **f**, **g** on gastric ulcer induced by HCl/ethanol solution are shown in Table 3. Oral administration of the ulcerogenic agent to the control group clearly showed a mucosal damage characterized by multiple haemorrhage red bands of different sizes along the long axis of the glandular stomach as described in other studies (Shay *et al.*, 1945; Yassir *et al.*, 1999). When we compared the gastroprotective activity of compounds **5a**, **b**, **f**, **g** we observed that

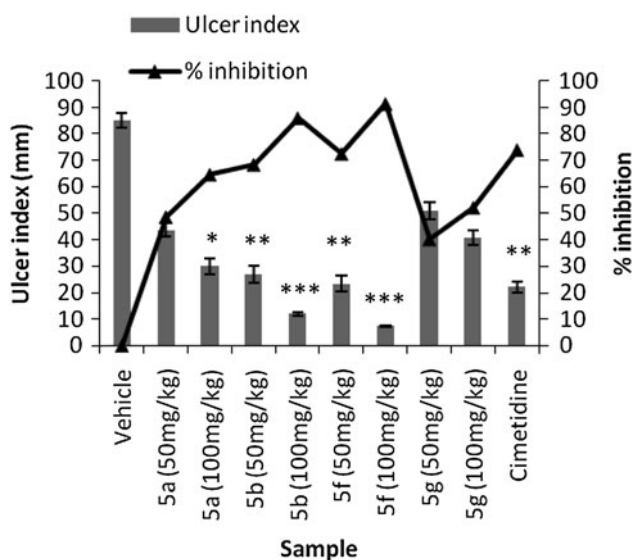


Fig. 3 Effect of compounds **5a**, **b**, **f**, **g** and the reference drug (cimetidine) on gastric ulcer induced by HCl/ethanol in rats. Data expressed as mean ± SEM ($n = 6$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from control group

Table 3 Effect of compounds **5a**, **b**, **f**, **g** and the reference drug (cimetidine) on gastric ulcer induced by HCl/ethanol in rats

Treatment	Dose (mg/kg)	Ulcer index (mm)	Inhibition (%)
Vehicle (2.5 ml/kg) (control)	–	85 ± 2.82	–
Compounds			
5a	50	43.66 ± 2.58	48.63
	100	30 ± 3.03*	64.7
5b	50	26.83 ± 3.43**	68.43
	100	11.83 ± 0.75***	86.08
5f	50	23.34 ± 2.9**	72.53
	100	7.29 ± 0.3***	91.42
5g	50	50.81 ± 3.2	40.22
	100	40.65 ± 2.8	52.17
Cimetidine (reference drug)	100	22.07 ± 2.12**	74.03

Data expressed as mean ± SEM ($n = 6$)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from control group

pyrazolopyrimidopyrimidine **5b** (100 mg/kg) demonstrated the higher significant inhibition of gastric lesion (91, 42 %).

In conclusion, we have synthesized a new series of 1,7-dihydropyrazolo [3',4':4,5]pyrimido[1,6-*a*]pyrimidine **5a–i** derivatives. The yield of the reaction seems to be significantly influenced by the nature of substituent. The highest yield is obtained for more hydrogen atom substituent. However, test (or experimental) compounds **5a, b, f** showed that the methyl group increases the anti-inflammatory activity, contrary to ethyl group which decreases this activity. The same interpretation is found with gastroprotective effect. Indeed, our results on the gastroprotective effects of compounds **5a, b, f** compared with cimetidine indicate that replacement of hydrogen by methyl reduces the gastrointestinal adverse effects.

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