



SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF SOME NEW PYRAZOLO[3,4-*d*] PYRIMIDINES

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Keywords: Pyrazolo[3,4-*d*]pyrimidinone, 6-aryaminomethyl derivatives, sulfa drug, tetrazole, anti-inflammatory activity.

5-Amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**5**) was reacted with chloroacetyl chloride under fusion condition to afford two compounds, which identified as 6-chloromethylpyrazolo[3,4-*d*]pyrimidine (**3**) and 4-amino-5-chloropyrazolo[3,4-*b*]pyridine (**7**) in different ratios. Treatment of compound (**3**) with aromatic amines, gave the arylaminomethyl derivatives (**8a-c**). Compound (**3**) was reacted with sodium azide, phosphorous oxychloride, sulphanilamide to give new derivatives (**9**, **10**, and **14**). The 4-chloro derivative (**11**) underwent nucleophilic substitution using some reagent such as P₂S₅, sodium azide and hydrazine hydrate to furnish novel pyrazolo[3,4-*d*]pyrimidines (**12**, **13**, **15**, **16**, **17**). All synthesized compounds were characterized using elemental analysis and spectral techniques. The anti-inflammatory activity of all the newly synthesized compounds was evaluated using the carrageenan induced paw oedema test in rats using indomethacin as the reference drug.

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Starting precursor, 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile (**5**), was prepared using the reported procedure.²⁵

Synthesis of 6-chloromethyl-1-phenyl-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (3**) and 4-amino-5-Chloro-1-phenyl-1,7-dihydro-pyrazolo[3,4-*b*]pyridin-6-one (**7**)**

A mixture of compound (**5**) (1 g, 5.4 mmol) and chloroacetyl chloride (0.75 mL, 6.6 mmol) was refluxed on water bath for 4 h. The reaction mixture was cooled and then heated with 15 mL of ethanol absolute for 5 min, the solid product was filtered, washed with hot ethanol, dried and recrystallized from DMF and identified as compound (**3**). The filtrate was then poured into ice cold water to give another solid, which was filtered off, dried and crystallized from dioxane and identified as compound (**7**).

Compound (**3**): Yield: 1.1 g (75.09 %), m.p. 272-274 °C. IR (cm⁻¹): 3150 (NH), 2988 (CH-aliphatic), 1672 (C=O). MS (*m/z* %): 260.41 (M⁺, 100). ¹HNMR (DMSO-*d*₆) δ = 12.75 (s, 1H, -NH), 8.50 (s, 1H, CH pyrazole), 8.25-7.45 (m, 5H, ArH), 4.36 (s, 2H, CH₂). Anal. Calcd. for C₁₂H₉ClN₄O (260.68): C, 55.29, H, 3.48, Cl, 13.60, N, 21.49 %. Found: C, 55.98, H, 3.26, Cl, 13.31, N, 21.18 %.

Compound (**7**): Yield: 0.35 g (75.12 %), m.p. 159-161 °C. IR (cm⁻¹): 3151, 3231 (NH, NH₂), 1679 (C=O). ¹HNMR (DMSO-*d*₆): δ = 11.48 (s, 2H, NH₂), 10.54 (s, 1H, -NH), 8.69 (s, 1H, CH pyrazole), 8.35-7.44 (m, 5 H, ArH and). Anal. Calcd. for C₁₂H₉ClN₄O (260.68): C, 55.29, H, 3.48, Cl, 13.60, N, 21.49%. Found: C, 55.63, H, 3.33, Cl, 13.73, N, 21.32 %.

General procedure for the synthesis of 6-arylaminoethyl-1-phenyl-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (8a-c**)**

To a solution of compound (**3**) (2 g, 7.6 mmol) in dioxane (30 mL), a substituted aniline (7.8 mmol) was added. The reaction mixture was heated under reflux for 3-5 h. The

Introduction

Pyrazole derivatives are important intermediates¹⁻⁵ that possess biological and pharmacological activities.⁶⁻¹⁰ The chemistry of pyrimidine and fused pyrimidine derivatives has been of increasing interest, since molecules based on the pyrazolo[3,4-*d*] pyrimidine ring system exhibit a multitude of interesting pharmacological properties including purine analogues,¹¹⁻¹³ CNS depressant,¹⁴ neuroleptic,¹⁵ tuberculostatic,¹⁶ antibacterial and antifungal.¹⁷ Pyrazolo[3,4-*d*]pyrimidines were also identified as a general class of adenosine receptors.¹⁸ Furthermore, pyrazolo[3,4-*d*]pyrimidine derivatives were found to possess anti-inflammatory,¹⁹ antiviral,²⁰ and antitumor activities.²¹ As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds,²²⁻²⁴ we now wish to report the synthesis of some new pyrazolo[3,4-*d*]pyrimidine derivatives to evaluate their anti-inflammatory activity by carrageenan paw edema method.

Experimental

Melting points are uncorrected and determined using a Gallenkamp melting point apparatus. The IR spectra were recorded on a Shimadzu 470 IR spectrometer (KBr) v max cm⁻¹. The ¹H and ¹³C NMR spectra were measured on a Varian EM-200 (1H: 400 MHz, 13C: 100 MHz) spectrometer with TMS as internal standard and DMSO-*d*₆ as a solvent and reported as δ ppm. Mass spectra were determined on a JEOL JMS-600 spectrometer. Elemental analyses (C, H, N, and S) were performed on an elemental analysis system GmbH VarioEL V2.3. The results were found to be in good agreement (±0.4 %) with the calculated value.

solvent was evaporated under reduced pressure and the residue was treated with water. The solid product was filtered off, washed with water, dried and recrystallized from ethanol.

6-Phenylaminomethyl-1-phenyl-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (8a)

Yield: 1.60 g (66.13 %), m.p. 189–191 °C. IR (cm⁻¹): 3398, 3275 (NH), 2990 (CH-aliphatic), 1682 (C=O). MS (*m/z* %): 317.30 (M⁺, 100). ¹HNMR (DMSO-*d*₆) δ = 12.30 (s, H, NH), 8.35–7.11 (m, 11H, ArH and CH-pyrazole), 5.98 (s, 1H, NH), 4.30 (s, 2H, CH₂). Anal. Calcd. for C₁₈H₁₅N₅O (317.34): C, 68.13, H, 4.76, N, 22.07 %. Found: C, 67.87, H, 4.13, N, 21.91 %.

6-(4-Chlorophenylamino)methyl-1-phenyl-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (8b)

Yield: 1.82 g (69.11 %), m.p. 229–231 °C. IR (cm⁻¹): 3350, 3189 (NH), 2950 (CH-aliphatic), 1677 (C=O). MS (*m/z* %): 350.98 (M⁺, 100). ¹HNMR (DMSO-*d*₆) δ = 12.35 (s, H, NH), 8.35–7.15 (m, 10H, ArH and CH-pyrazole), 6.09 (s, 1H, NH), 4.45 (s, 2H, CH₂). Anal. Calcd. for C₁₈H₁₄ClN₅O (351.79): C, 61.46, H, 4.01, Cl, 10.08, N, 10.91 %. Found: C, 61.32, H, 3.89, Cl, 9.92, N, 19.88 %.

6-(4-Tolylamino)methyl-1-phenyl-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (8c)

Yield: 1.29 g (51.07 %), m.p. 191–193 °C. IR (cm⁻¹): 3330, 3170 (NH), 2955 (CH-aliphatic), 1690 (C=O). ¹HNMR (DMSO-*d*₆) δ = 11.25 (s, H, NH), 8.80 (s, 1H, CH-pyrazole), 8.30–7.33 (m, 9H, ArH), 6.11 (s, 1H, NH), 4.40 (s, 2H, CH₂). Anal. Calcd. for C₁₈H₁₄ClN₅O (331.38): C, 68.87, H, 5.17, N, 21.13 %. Found: C, 68.49, H, 5.03, N, 20.94 %.

6-(Azidomethyl)-1-phenyl-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (9)

To a solution of compound (3) (1 g, 3.8 mmol) in DMF (20 mL), sodium azide (0.9 g, 14 mmol) was added. The reaction mixture was heated under reflux for 10 h. After cooling, 20 mL of cold water was added and extracted with diethyl ether (3x10 mL). The organic layers were combined, washed thoroughly with water (3x10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure, a yellowish orange crystals was obtained, which was recrystallized from dioxane-H₂O (1:1). Yield: 1.29 g (51.07 %), m.p. 191–193 °C. IR (cm⁻¹): 3290 (NH), 2990 (CH-aliphatic), 2235 (N₃), 1685 (C=O). MS (*m/z* %): 211.17 (M⁺-CH₂N₃⁺, 65). ¹HNMR (DMSO-*d*₆) δ = 11.95 (s, H, NH), 8.40 (s, 1H, CH-pyrazole), 8.25–7.23 (m, 5 H, ArH), 3.35 (s, 2H, CH₂). Anal. Calcd. for C₁₂H₉N₇O (267.25): C, 53.93, H, 3.39, N, 36.69 %. Found: C, 52.57, H, 3.18, N, 36.31 %.

4-Chloro-6-(chloromethyl)-1-phenyl-pyrazolo[3,4-*d*]pyrimidine (10)

A mixture of (3) (1 g, 3.8 mmol) and phosphorus oxychloride (2 mL, 22 mmol) was refluxed at 115 °C for 5h, cooled and poured onto ice water with stirring. The dark

purple powder was collected and recrystallized from acetone. Yield: 1.06 g (95.13 %), m.p. 110–112 °C. IR (cm⁻¹): 2990 (CH-aliphatic), MS (*m/z* %): 278.02 (M⁺, 100). ¹HNMR (DMSO-*d*₆) δ = 8.45 (s, 1H, CH-pyrazole), 8.15–7.42 (m, 5 H, ArH), 4.97 (s, 2H, CH₂). Anal. Calcd. for C₁₂H₈Cl₂N₄ (279.12): C, 51.64, H, 2.89, Cl, 25.40, N, 20.07 %. Found: C, 51.39, H, 3.12, Cl, 25.28, N, 20.41 %.

4-Chloro-N-[(4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)methyl]benzenamine (11)

Method A

A mixture of (10) (1.30 g, 5 mmol) and 4-chloroaniline (0.69 g, 5.20 mmol) in dioxane was refluxed for 5h, cooled and poured onto iced water with stirring. A light purple solid was collected and recrystallized from ethanol. Yield: 1.45 g (85.13 %), m.p. 195–197 °C. IR (cm⁻¹): 3354 (NH), 2995 (CH aliphatic), MS (*m/z* %): 369.00 (M⁺-H, 66.12). ¹HNMR (DMSO-*d*₆) δ = 8.60 (s, 1H, CH-pyrazole), 8.04–6.95 (m, 9H, ArH), 5.70 (s, 1H, NH), 3.95 (s, 2H, CH₂). Anal. Calcd. for C₁₈H₁₃Cl₂N₅ (370.24): C, 58.39, H, 3.54, Cl, 19.15, N, 18.92 %. Found: C, 58.68, H, 3.47, Cl, 19.56, N, 18.71 %.

Method B

A mixture of (8b) (0.35 g, 1 mmol) and phosphorus oxychloride (0.5 mL, 5.5 mmol) was refluxed at 115°C for 5h. The cooled reaction mixture was poured into water (20 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol to give (10). It was identical with the product obtained by method A (m.p. and mixed m.p.). Yield: 0.26 g (72.31 %).

6-[(4-Chlorophenylamino)methyl]-N-(4-chlorophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (12)

A mixture of (10) (1.30 g, 5 mmol) and 4-chloroaniline (1.39 g, 10.40 mmol) in dioxane was refluxed for 10 h, cooled and poured onto iced water with stirring. A light brown solid was collected and recrystallized from ethanol-water (1:1). Yield: 1.42 g (66.24 %), m.p. 255–257 °C. IR (cm⁻¹): 3354, 3220 (NH), 2985 (CH aliphatic), MS (*m/z* %): 460.88 (M⁺-1, 59.11). ¹HNMR (DMSO-*d*₆) δ = 8.50 (s, 1H, CH-pyrazole), 8.21–7.05 (m, 13 H, ArH), 5.66 (s, 1H, NH), 3.55 (s, 2H, CH₂). Anal. Calcd. for C₂₄H₁₈Cl₂N₆ (461.35): C, 62.48, H, 3.93, Cl, 15.37, N, 18.22 %. Found: C, 62.83, H, 3.52, Cl, 15.61, N, 18.11 %.

6-[(4-Chlorophenylamino)methyl]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (13)

A mixture of (8b) (0.70 g, 2 mmol) and phosphorus pentasulfide (0.5 g, 2.30 mmol) in dry pyridine (10 mL) was refluxed for 12 h, cooled and poured into iced water, acidified to pH 4–5. A yellow powder was separated. The precipitate was filtered, washed thoroughly with water, dried and recrystallized from ethanol. Yield: 0.58 g (80.19 %), m.p. 161–163 °C. IR (cm⁻¹): 3330, 3286 (NH), 2916 (CH aliphatic), 1085 (C=S). ¹HNMR (DMSO-*d*₆) δ = 12.29 (s, 1H, NH), 8.45 (s, 1H, CH-pyrazole), 8.27–7.09 (m, 10 H, ArH), 4.70 (s, 2H, CH₂), 3.90 (s, 1H, NH). Anal. Calcd. for

$C_{18}H_{14}ClN_5S$ (367.86): C, 58.77, H, 3.84, Cl, 9.64, N, 19.04, S, 8.72 %. Found: C, 59.12, H, 3.67, Cl, 9.43, N, 19.25, S, 8.48 %.

4-[(4-Oxo-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)methyl]amino}benzenesulfonamide (14)

A mixture of (3) (0.5 g, 1.9 mmol) and sulfanilamide (0.35 g, 2 mmol), in dry pyridine (10 mL), was refluxed for 3 h, cooled and poured into cold water with stirring, filtered off and the filtrate concentrated, cooled in a refrigerator, a light orange powder was obtained, which was recrystallized from dioxane. Yield: 0.48 g (64.27 %), m.p. 315–317 °C. IR(cm^{-1}): 3410, 3390, 3270 (NH, NH₂), 2972 (CH aliphatic), 1360, 1180 (S=O). MS (m/z %): 392.08 (M⁺-4H, 33.38). ¹HNMR (DMSO-*d*₆) δ = 13.15 (s, 1H, NH pyrimidine), 8.65 (s, 1H, CH-pyrazole), 9.20 (s, 1H, NH-SO₂), 8.45-7.29 (m, 9H, ArH), 6.11 (s, 1H, NH₂), 3.34 (s, 2H, CH₂). Anal. Calcd., for $C_{18}H_{16}N_6O_3S$ (396.42): C, 54.54, H, 4.07, N, 21.20, S, 8.09 %. Found: C, 54.76, H, 4.29, N, 21.03, S, 8.19 %.

4-Hydrazino-N-[(4-chloro-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)methyl]benzenamine (15)

A mixture of (11) (0.74 g, 2 mmol) and hydrazine hydrate (5 mL) in absolute ethanol (20 mL) was refluxed for 6 h. The reaction mixture was poured onto ice. The product was isolated and crystallized from acetic acid as reddish white needles. Yield: 0.49 g (68.31 %), m.p. 225–227 °C. IR (cm^{-1}): 3450, 3375, 3260 (NH, NH₂), 2997 (CH aliphatic). ¹HNMR (DMSO-*d*₆) δ = 12.75 (s, 1H, NH), 10.85 (s, 1H, NH), 8.50 (s, 1H, CH pyrazole), 8.27-7.11 (m, 9 H, ArH), 4.95 (s, 2H, NH₂), 3.80 (s, 2H, CH₂). Anal. Calcd. for $C_{18}H_{16}ClN_7$ (365.82): C, 59.10, H, 4.41, Cl, 9.69, N, 26.80 %. Found: C, 59.38, H, 4.19, Cl, 9.81, N, 26.62 %.

4-Azido-N-[(4-chloro-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)methyl]benzenamine (16)

A mixture of (11) (0.74 g, 2 mmol) and sodium azide (0.13 g, 2 mmol) in DMF (20 mL) was stirred at room temperature overnight. The reaction mixture was extracted with CH₂Cl₂ (3x10 mL) and the organic layer was dried over anhydrous CaCl₂. The solvent was removed under reduced pressure to give a light brown solid and recrystallized from ethanol. Yield: 0.52 g (70.18 %), m.p. 118–120 °C. IR (cm^{-1}): 3250, (NH), 2980 (CH aliphatic), 2202 (N₃). ¹HNMR (DMSO-*d*₆) δ = 8.60 (s, 1H, CH-pyrazole), 8.15-7.15 (m, 9 H, ArH), 5.90 (s, 1H, NH), 3.78 (s, 2H, CH₂). Anal. Calcd., for $C_{18}H_{13}ClN_8$ (376.80): C, 57.38, H, 3.48, Cl, 9.41, N, 29.74 %. Found: C, 57.09, H, 3.82, Cl, 9.24, N, 29.93 %.

4-Chloro-N-[(7-phenyl-7H-pyrazolo[4,3-*e*]tetrazolo[1,5-*c*]pyrimidin-5-yl)methyl]aniline (17)

Method A

A mixture of (11) (0.37 g, 1 mmol) and sodium azide (0.06 g, 1 mmol) in acetic acid (10 mL) was refluxed for 8 h. The reaction mixture was cooled and diluted with water and extracted with CH₂Cl₂ (3x10 mL) and the oil layer dried over anhydrous Na₂SO₄. The solvent was removed under

reduced pressure to give a pale brown solid, which was recrystallized from dioxane. Yield: 0.25 g (66.10 %), m.p. 168–170 °C. IR (cm^{-1}): 3320, (NH), 2890 (CH aliphatic), 1281, 1136 (N=N=N). MS (m/z %): 348.59 (M⁺-N₂, 71.29). ¹HNMR (DMSO-*d*₆) δ = 8.67 (s, 1H, CH-pyrazole), 8.19-7.08 (m, 9 H, ArH), 6.12 (s, 1H, NH), 4.11 (s, 2H, CH₂). Anal. Calcd. for $C_{18}H_{13}ClN_8$ (376.80): C, 57.38, H, 3.48, Cl, 9.41, N, 29.74 %. Found: C, 57.55, H, 3.67, Cl, 9.73, N, 29.51 %.

Method B

A solution of sodium nitrite (0.07 g, 1 mmol) dissolved in the least amount of water was added drop wise to an ice-cold solution of compound (15) (0.36 g, 1 mmol) in acetic acid (10 mL) and kept in an ice bath at -5 °C. The reaction mixture was allowed to stand overnight at room temperature, then it was poured into water (100 mL). The precipitate that formed was filtered off and crystallized from dioxane to give (17). It was identical with the product obtained by method A (m.p. and mixed m.p.). Yield: 0.22 g (58.42 %).

Anti-inflammatory evaluation

Adult albino rats, weighing 150–200 g, were used. The animals were allowed food and water ad libitum, except during the experiment. They were housed in a room at 23±2 °C with a 12 h light/dark cycle. The animals were randomly allocated into groups of 6 animals each at the beginning of the experiment and were fasted for 24 h before the experiment with free access to water. All of the compounds and the reference drug were suspended in a 0.5 % carboxymethyl cellulose (CMC) solution. The standard drug indomethacin was administered orally at a dose of 20 μ mol kg⁻¹. The tested compounds were administered orally at an equimolar oral dose relative to 20 μ mol kg⁻¹ of indomethacin. The control group received a 0.5 % CMC solution. Into the sub-plantar region of the right hind paw of each rat, 0.1 mL of 1 % carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds and standard drug. The right paw volume was measured using a digital plethysmometer (Model 7150, Ugo Basile, Varese, Italy), directly before and after 1, 2, 3 h, intervals after administration of the tested compounds. The percent oedema inhibition was calculated from the mean effect in the control and treated animals according to the following equation.

$$\text{Percent oedema inhibition} = (v_c - v_t/v_c) \times 100 \quad (1)$$

where

v_t represents the mean increase in paw volume in rats treated with tested compounds and

v_c represents the mean increase in paw volume in the control group of rats.

Potency was expressed as % oedema inhibition of the tested compounds relative to % oedema inhibition of indomethacin as depicted in Table 1. All the results are expressed as the mean±standard error of the mean (S.E.M.). Statistical evaluation was performed using A NOVA

Results and Discussions

The only synthesis report²² available for the key intermediate compound (**3**) was route A in Scheme 1, in which readily available compound (**1**) reacted²⁵ with chloroacetyl chloride to yield acylated product (**2**), which underwent subsequent ring closure to produce (**3**).

Table 1. Anti-inflammatory activity of some pyrazolo[3,4-*d*]pyrimidine derivatives using acute carrageenan-induced paw oedema in rats.

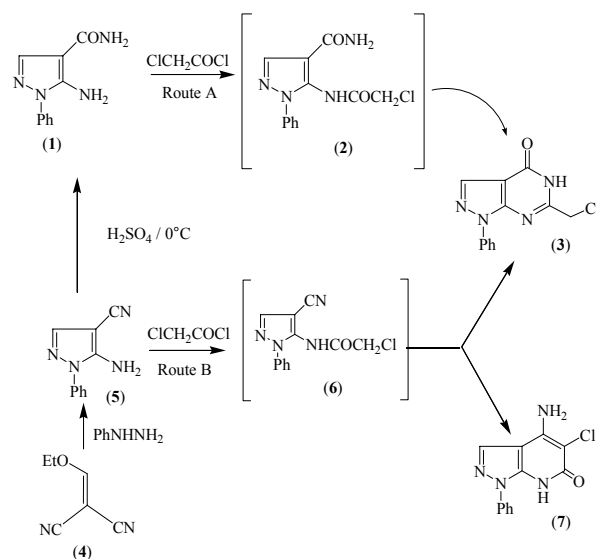
Compd	Oedema inhibition ^a			Potency
	1 h	2 h	3 h	
8a	12.2±1.4	16.7 ± 1.2	24.5±1.5	41.6
8b	32.2±1.0	44.1 ± 1.5	60.6 ±1.2	>100
8c	9.6 ±1.5	13.3 ± 1.3	19.2 ±1.5	32.6
9	12.3 ±1.1	19.6 ± 1.3	24.8 ±0.8	42.2
10	36.4±1.6	48.3± 1.1	63.2± 0.9	>100
11	33.8±1.4	42.3± 1.3	60.8±1.1	>100
12	22.6±1.3	27.2± 1.4	38.8± 1.4	65.9
13	37.2 ±1.0	46.1 ± 1.1	64.1± 1.5	>100
14	39.9 ±1.2	50.1 ± 1.3	68.9 ±1.2	>100
15	30.2 ±0.9	36.6 ± 1.1	39.1 ±1.1	66.4
16	27.3 ±1.4	29.2 ± 1.1	33.7 ±0.9	57.3
17	17.8 ±0.99	23.2 ± 1.1	29.3 ±1.1	49.8
Indo-methaci	31.5 ±1.2	40.2 ± 1.2	58.8 ±1.5	100.0

^aDose 20 μmol kg⁻¹, *n* = 6, Statistically significant from the indomethacin at *p*<0.05.

We were able to obtain the annulated products (**3**) and (**7**) following this literature method via route B in Scheme 1. Thus, when (**5**) was treated with chloroacetyl chloride and heating on steam bath for long time (Route B), two products (**3**) and (**7**) in 75 % and 25 % yield, respectively were isolated. The reaction mixture was separated by fractional crystallization, in which a crystalline solid, m.p. 272–274 °C (Lit, 270-272 °C)²² obtained from hot ethanol was characterized as (**3**) formed through Dimroth rearrangement mechanism. The second solid fraction m.p. 159–161 °C was obtained after cooling the filtrate and poured onto water, which was identified as (**7**). The structure of compound (**3**) was confirmed by the absence of the cyano group, and the presence of two bands at 3150 cm⁻¹ and at 1672 cm⁻¹ for both NH and C=O groups, respectively. Additionally, mass spectrum of compound **3** exhibited a molecular ion peak at 260.41 (M⁺, 100%), which is in agreement with the expected structure. (Scheme 1). The structure of (**7**) was elucidated using spectral and analytical analysis. Its ¹HNMR displayed two characteristic peaks at 11.48 and 10.54 ppm for both NH₂ and NH protons.

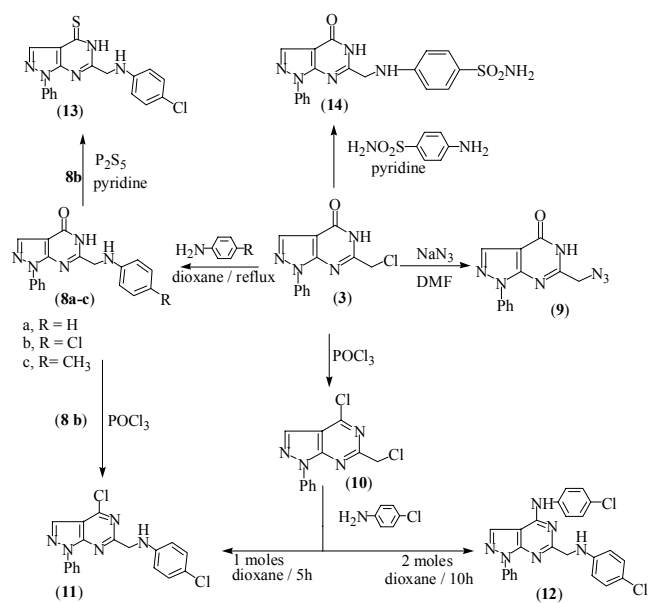
Pyrazolopyrimidine (**3**) underwent nucleophilic substitution reaction of chlorine atom with primary aromatic amines in dioxane to give (**8a-c**). Structure of compounds (**8a-c**) showed absorption bands in the range 3398-3170 cm⁻¹ for two NH groups and 1690-1677 cm⁻¹ for C=O group. Their ¹HNMR displayed the characteristic signals at 5.98-6.11 ppm for -NH, at 4.30-4.45 ppm for methylene protons CH₂ and at 12.30-11.25 ppm due to NH-pyrimidine. The mass spectrum of (**8a**) showed a molecular ion peak at 317.30 (M⁺, 100%), which is in agreement with the suggested structure. When compound (**3**) was heated with sodium azide in the presence of DMF, it afforded the

corresponding 6-azidomethyl derivative (**9**). The IR spectrum of (**9**) showed an absorption band at 2235 cm⁻¹ corresponding to N₃ function. Also, in the mass spectrum of (**9**), the molecular ion peak at *m/z* = 267.25 was not observed, but it showed a base peak at *m/z* = 211.17 due to loss of CH₂N₃⁺. Compound (**10**) was obtained by the chlorination of (**3**) using phosphorus oxychloride. The



Scheme 1. Synthesis of compounds (**3**) and (**7**).

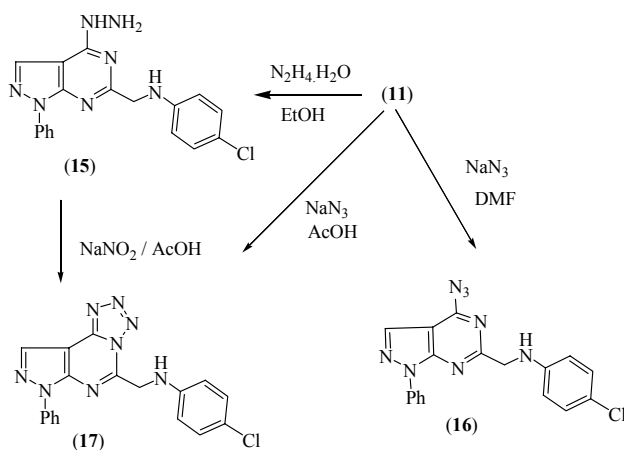
structure of (**10**) was confirmed by both analytical and spectral data. Thus, IR spectrum revealed the disappearance of absorption bands at 3150 and 1672 cm⁻¹ for NH and C=O. MS of (**10**) exhibited a molecular ion peak at 278.02 (M⁺, 100%), which is in agreement with the expected structure. Regioselective amination reactions of compound (**10**) using one and two equivalents of 4-chloroaniline in refluxing dioxane, indicated that, the reaction products depends on the molar ratios and the duration of reaction. Thus, refluxing of (**10**) with one mole for 5 h, afforded the product of the most replaceable sp³ chlorine atom, (**11**).



Scheme 2. Synthesis of compounds (**8** – **14**) from (**3**).

As a chemical evidence, the formation of compound (**11**) was also achieved through heating of a sample of (**8b**) with phosphorus oxychloride. On the other hand, treatment of (**10**) with two moles of 4-chloroaniline for 8 h, yielded the (**12**). The structures of (**11**) and (**12**) were deduced from their satisfactory spectral and analytical data, for example, the MS of compound (**11**) showed its correct parent ion peak at 369.0 (M^+ , 100 %) 1H NMR spectrum showed two characteristic peaks at 5.70 ppm for NH proton. The pyrazolopyrimidinone derivative (**8b**) was converted into the corresponding thione (**13**) by refluxing with P_2S_5 in dry pyridine. As an example for sulfa drugs, the derivative (**14**) was synthesized by refluxing of compound (**3**) with sulphanilamide in dry pyridine. The IR spectrum of compound (**14**), showed absorption bands at 3410, 3390, 3270 and 1360 cm^{-1} due to NH, NH_2 and SO_2 groups (Scheme 2).

Finally, the behavior of compound (**11**) towards some nucleophilic reagent was investigated. Thus, treatment of (**11**) with hydrazine hydrate in boiling ethanol, furnished the corresponding 4-hyrazino derivative (**15**) in good yield. The structure of compound (**15**) was elucidated from its elemental and spectral data. The IR spectrum showed absorption bands at 3450, 3375 and 3260 cm^{-1} assignable to NH- NH_2 group. Also, its 1H NMR spectrum showed the presence of the three NH protons at 12.75, 10.85 and 4.95 ppm. Furthermore, interaction of (**11**) with sodium azide in DMF as a basic solvent, furnished the 4-azido derivative (**16**), whereas upon heating in acetic acid, the pyrazolotetrazolopyrimidine derivative (**17**) was obtained via ring closure reaction with the N3-pyrimidine ring.²⁶ As a chemical evidence, the formation of compound (**17**) was achieved through heating of (**15**) with sodium nitrite in acetic acid at 0 °C. The structure of compounds (**16**) and (**17**) was confirmed using elemental and spectral data. The IR spectrum of compound **16** revealed absorption bands at 3250 and 2202 cm^{-1} for both NH and N_3 (azido) groups, respectively. The disappearance of the absorption band for N_3 group in compound (**17**), which displayed characteristic new absorption bands at 1281, 1136 for N-N=N (tetrazole ring). In the MS of (**17**), the molecular ion peak at $m/z = 376.80$ was not observed, but it showed a base peak at $m/z = 348.59$ due to loss of N_2^+ , reinforcing the formation of (**17**) (Scheme 3).



Scheme 3. Synthesis of compounds (**15** – **17**) from (**11**).

Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds was determined by the acute carrageenan induced paw oedema standard method in rats.²⁷ From the obtained results (Table 1), it has been observed that several newly prepared compounds (**8b**, **10**, **11** and **13**) revealed better anti-inflammatory properties (60.6–64.1 % inhibition of oedema) as compared to that by indomethacin (58.8 % inhibition). In addition, substitution in compound (**3**) with 4-aminobenzenesulphonamide group resulting in (**14**), which is the most effective anti-inflammatory agent, revealing better activity (68.9 % inhibition) than that of indomethacin. However, comparing the activity of 6-arylamino-methyl-1-phenyl-pyrazolo[3,4-d]pyrimidin-4-one (**5H**)-ones (**8a-c**), it was observed that that substitution with an electron-withdrawing group, chloro, in phenyl (**8b**) (60.6 % inhibition), seems more favorable for constructing an anti-inflammatory active agent than the case of substitution with an electron-donating group CH_3 , as exhibited in (**8c**) (19.2 % inhibition). Meanwhile, upon chlorination of compound (**3**), the obtained 4-chloro derivative (**10**) showed a significant increase in the anti-inflammatory (63.2 % inhibition). However, replacement of the chlorine atom of compound (**10**) in position 6 by 4-chloroaminophenyl group derivative (**11**) possessed higher activity (60.8 % inhibition) than the di-substituted isomer (**12**) (38.8 % inhibition). Finally, (**13**) exhibited more anti-inflammatory activity (64.1 % inhibition) than its ketone analogue (**8b**), comparable to that of indomethacin (58.8 % inhibition). Among the synthesized compounds (**15-17**), no significant difference was perceived in the activity and they showed weak to moderate activity (49.8 - 66.4 % inhibition).

Conclusion

The objective of the present study was to synthesize, characterize and investigate the anti-inflammatory activity of some new pyrazolo[4,3-d]pyrimidine derivatives. The starting compound 6-chloromethyl-1-phenyl-1,5-dihydro-pyrazolo[3,4-d]pyrimidin-4-one (**3**) was used to synthesize the target compounds. Compounds (**8b**, **10**, **11**, **13** and **14**) showed the most anti-inflammatory activity when compared to that of indomethacin.

Acknowledgments

The authors are grateful to Prof. Dr. Ahmed Moharem, Professor of Botany and Microbiology department and the director of Mycology center and colleagues of Faculty of Medicine, Assiut University for their kind help in performing the pharmacological screening. **Conflict of Interest:** The authors declare no conflict of interest.

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Received: 17.09.2016.

Accepted: 04.10.2016.