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Synthesis, Structural Characterization of Some Pyrazolo [3,4-*d*] pyrimidine Derivatives as Anti-inflammatory Agents

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A SERIES of pyrimidine and fused triazolopyrimidine derivatives were newly synthesized using aminopyrazole carbonitrile 1 as a had starting material and compounds 14 and 16 are intermediates. Initially, the acute toxicity of the compounds was assayed *via* the determination of their LD_{50}, and all compounds were interestingly less toxic and had lower ulcerogenic activities than Diclophenac® as a reference drug. Regarding the protection against Carrageenan induced edema; the pharmacological screening showed that compounds 17, 11, 13 and 5 have good anti-inflammatory activities comparable to the reference drug. On the other hand, in searching for COX-2 inhibitor, the inhibition of plasma prostaglandine (PGE_2) for the compounds was determined and the same four compounds were found the more potent agents. The detailed synthesis, spectroscopic data, pharmacological screening and acute toxicity LD_{50} for the synthesized compounds were reported.

Keywords: Pyrazole, Pyrimidine and Anti-inflammatory .

Non-steroidal anti-inflammmatory drugs (NSAIDs) have been used to treat various ailments for over 100 years. As a class, these drugs possess anti-inflammatory, anti-allergy, analgesic and antipyretic activity and are widely used to treat chronic inflammatory states such as arthritis, psoriasis and asthma. All of NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects (like in gastrointestinal), in a significant fraction of treated patients and this frequently limits therapy [(1)].

Pyrazolo[3,4-*d*]pyrimidines are a class of naturally occurring fused uracils that possess a wide range of biological activity[(2,3)]. An example of these biologically active isomeric purine analogues is allopurinol (pyrazolo [3,4-*d*] pyrimidin-4-one), which inhibits xanthine oxidase and subsequently is used for the treatment of hyperuracemia and gouty arthritis[(4)]. Furthermore, the allopurinol 1-ribo-nucleotide-like 6-azauridine-5`-

phosphate is a potential antiviral and antitumor agent. Several reports are available which suggest that 4-aminopyrazolo [3,4-*d*] pyrimidine nucleosides and related compounds may function as substrates for anabolic and catabolic enzymes[5-9]. Recently, it was reported that some pyrazolo [3,4-*d*]pyrimidine derivatives as a new class of ant-inflammatory and anti-platelet agents[10]. Starting from these observation and in continuation to our interest in the synthesis of pyrazolo [3,4-*d*] pyrimidines[11] and exploring novel anti-inflammatory candidates[12-14], we report herein the synthesis and pharmacological evaluation of some new compounds conserving the pyrazolo [3,4-*d*] pyrimidine nucleus.

Results and Discussion

5-Amino-3-methylthio-1-(4`-nitrophenyl)pyrazol-4-carbonitrile (1), the key starting material of the present investigation, was prepared in good yield by stirring a mixture of 1,1`-dicyano-2,2`-dithiomethylmethylene and 4-nitrophenylhydrazine in dioxane and a catalytic amount of piperidine, according to Tominaga *et al.* method[15] (Scheme 1). The reaction of 1 with formamide in the presence of acetic anhydride under reflux afforded the fused 4-aminopyrimidine 2 (Table 1). The ^{1}H NMR showed the CH-pyrimidine signal at δ= 8.69 ppm and its IR spectrum showed the absence of a C≡N group band present in 1 and the presence of the amino group as indicated by bands at ν= 3417, 3325 cm^{-1} (Table 2).

Compound 1 reacted with formic acid to afford the pyrazolo[3,4-*d*]pyrimidin-2-one derivative 3. The structure of compound 3 was confirmed with spectral data, where its IR spectrum showed bands at ν= 3227 cm^{-1} (NH) and at ν=1669 cm^{-1} (C=O), its ^{1}H NMR spectrum showed signals at δ= 7.79 and 8.1 ppm for the pyrimidine and NH protons, respectively. Also, its mass spectrum showed the molecular ion peak (M^+, 34%) at m/z= 303 corresponding to its molecular formula $C_{12}H_9N_5O_3S$. Meanwhile, 1 was converted to the pyrimidine-4(5*H*)-thione derivative 4 when subjected to the action of triethyl orthoformate in the presence of acetic anhydride followed by treatment with an ethanolic solution of sodium hydrogen sulfide[16]. The products obtained from the reaction of 1 with carbon disulfide are varied according to the reaction medium. In pyridine, the 6-imino-1,3-thiazine-2(1*H*)-thione (5) was formed, whereas in aqueous potassium hydroxide the pyrimidine dithione 6 was obtained. Under the action of aqueous potassium hydroxide, compound 5 was rearranged to 6 *via* a 1,3-thiazine-pyrimidine rearrangement in accordance with previous findings[17]. These results indicate that the formation of 6 proceeded *via* the 1,3-thiazine (5) which is irreversibly isomerized to the corresponding dithione under the reaction condition (Scheme 1).

TABLE 1. Physical data of the prepared compounds 1-13, 15 and 17 .

Compd. No.	Yield	Mp°C solvent	Mol. Formula (M. Wt.)	Elemental Analysis (Calcd/ Found)			
				C	H	N	S
1	85	232-234 dioxane	$C_{11}H_9N_5O_2S$ (275.29)	47.99 47.95	3.30 3.27	25.44 25.53	11.65 11.62
2	53	265-267 dioxane/ H_2O	$C_{12}H_{10}N_6O_2S$ (302.31)	47.68 47.76	3.33 3.37	27.80 27.68	10.61 10.67
3	75	273-275 dioxane	$C_{12}H_9N_5O_3S$ (303.30)	47.52 47.58	2.99 3.04	23.09 22.98	10.57 10.64
4	87	287-289 AcOH	$C_{12}H_9N_5O_2S_2$ (319.36)	45.13 45.21	2.84 2.88	21.93 22.85	20.08 20.16
5	69	249-251 dioxane/ H_2O	$C_{12}H_9N_5O_2S_3$ (351.43)	41.01 41.11	2.58 2.66	19.93 19.83	27.37 27.46
6	59 (A) 65 (B)	301-303 AcOH / H_2O	$C_{12}H_9N_5O_2S_3$ (351.43)	41.01 41.09	2.58 2.59	19.93 19.87	27.37 27.41
7	85	172-174 EtOH	$C_{14}H_{13}N_5O_3S$ (331.26)	50.75 50.67	3.95 3.92	21.14 21.23	9.68 9.62
8	65	233-235 (d) EtOH / H_2O	$C_{12}H_{11}N_7O_2S$ (317.33)	45.42 45.48	3.49 3.54	30.90 30.78	10.10 10.18
9	95 (A) 67 (B)	296-298 DMF	$C_{12}H_{11}N_7O_2S$ (317.33)	45.42 45.34	3.49 3.45	30.90 31.03	10.10 10.16
10	84	205-207 EtOH	$C_{12}H_8ClN_5O_2S$ (321.74)	44.80 44.72	2.51 2.47	21.77 21.83	9.97 10.04
11	73 (A) 53 (B)	253-255 dioxane	$C_{13}H_9N_7O_2S$ (327.32)	47.70 47.84	2.77 2.83	29.95 29.83	9.80 9.72
12	65	216-218 EtOH	$C_{13}H_{11}N_7O_3S$ (345.34)	45.21 47.13	3.21 3.25	28.39 29.47	9.29 9.32
13	65	267-269 dioxane	$C_{13}H_9N_7O_3S$ (327.32)	47.70 47.62	2.77 2.71	29.95 30.07	9.80 9.92
15	59	224-226 dioxane/ H_2O	$C_{18}H_{12}N_8O_2S$ (404.41)	53.46 53.38	2.99 2.91	27.71 27.85	7.93 7.87
17	52	289-291 DMF/ H_2O	$C_{19}H_{14}N_8O_2S$ (418.43)	54.54 54.62	3.37 3.45	26.78 26.66	7.66 7.75

TABLE 2. Spectral data of the prepared compounds 1 - 13, 15 and 17 .

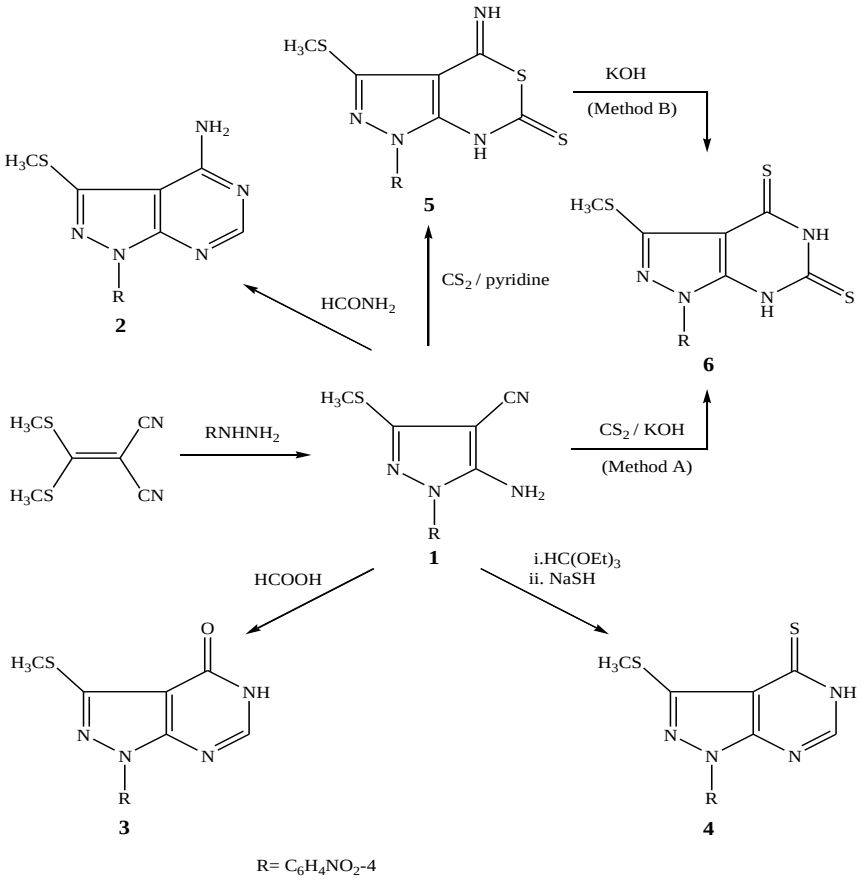
Compd. No.	IR ν (cm^{-1})	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)	MS m/z (*I*, %)
1	3360, 3300 (NH_2), 2200 (C≡N).	2.55 (s, 3H, SCH_3), 7.02 (bs, 2H, NH_2, D_2O-exchangeable), 8.1 (d, 2H, Ph), 8.45 (d, 2H, Ph).		275 (M^+,100).
2	3417, 3325 (NH_2).	2.59 (s, 3H, SCH_3), 5.74 (s, 2H, NH_2, D_2O-exchangeable), 7.8 (d, 2H, Ph), 8.23 (d, 2H, Ph), 8.69 (s, 1H, CH-pyrimidine).	14.4 (1C, SCH_3), 113.4 (1C, C_4), 125.2 (2C, Ph), 125.3 (2C, Ph), 143.8 (1C, C_3), 146.9 (1C, Ph), 147.8 (1C, Ph), 149.5 (1C, C_9), 157.9 (1C, CH-pyrimidine), 158.3 (1C, C-NH_2).	302 (M^+,100).
3	3227 (NH), 1679 (C=O).	2.57 (s, 3H, SCH_3), 7.83 (d, 2H, Ph), 8.19 (d, 2H, Ph), 8.52 (s, 1H, CH-pyrimidine), 10.43 (s, 1H, NH, D_2O-exchangeable).	13.4 (1C, SCH_3), 108.1 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 143.2 (1C, C_3), 143.6 (1C, C_9), 146.6 (1C, Ph), 146.8 (1C, CH-pyrimidine), 147.8 (1C, Ph), 161.8 (1C, C=O).	303 (M^+,100).
4	3370 (NH) 1183 (C=S).	2.57 (s, 3H, SCH_3), 7.82 (d, 2H, Ph), 8.21 (d, 2H, Ph), 8.78 (s, 1H, CH-pyrimidine), 9.3 (s, 1H, NH, D_2O-exchangeable).	14.4 (1C, SCH_3), 108.4 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 142.4 (1C, C_3), 143.6 (1C, C_9), 146.7 (1C, Ph), 146.9 (1C, CH-pyrimidine), 147.8 (1C, Ph), 181.3 (1C, C=S).	319 (M^+, 42), 275 (32) 216 (57) 122 (100).
5	3432 (NH), 3336 (NH) 1228 (C=S).	2.59 (s, 3H, SCH_3), 7.74-7.77 (m, 3H, Ph + NH,D_2Oexchangeable), 8.20 (d, 2H, Ph), 8.45 (s, 1H, NH, D_2O-exchangeable).	14.4 1c, SCH_3), 100 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 135.6 (1C, C_9), 137.5 (1C, C_3), 146.6 (1C, Ph), 147.2 (1C, Ph), 155.7 (C=NH), 193.6 (C=S).	350 (M^+, 3), 277 (14) 248 (35) 78 (100).
6	3367 (NH), 3343 (NH) 1206 (C=S).	2.59 (s, 3H, SCH_3), 7.65 (d, 2H, Ph), 8.01 (d, 2H, Ph), 8.45 (brs, 2H, 2 NH, D_2O-exchangeable).	15.4 (1C, SCH_3), 100.4 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 135.5 (1C, C_9), 137.5 (1C, C_3), 146.8 (1C, Ph), 147.8 (1C, Ph), 167.3 (1C, C=S), 178.8 (1C, C=S).	350 (M^+, 17), 229 (45) 78 (100).

TABLE 2. (Continued).

Compd. No.	IR ν (cm^{-1})	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)	MS m/z (*I*, %)
7	2218 (C≡N).	1.42 (t, 3H, CH_3), 2.54 (s, 3H, SCH_3), 4.34 (q, 2H, CH_2), 7.97 (d, 2H, Ph), 8.45 (d, 2H, Ph).		331 (M^+, 100).
8	3370, 3300 (NH_2), 3150 (NH).	2.55 (s, 3H, SCH_3), 4.95 (br s, 2H, NH_2, D_2O-exchangeable), 7.77 (d, 2H, Ph), 7.85 (s, 1H, =NH, D_2O-exchangeable), 8.17 (d, 2H, Ph), 8.23 (s, 1H, CH-pyrimidine).	14.6 (1C, SCH_3), 108.4 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 142.3 (1C, C_3), 143.6 (1C, C_9), 146.2 (1C, Ph), 146.3 (1C, Ph), 158.3 (1C, CH-pyrimidine), 161.4 (1C, C=NH).	318 (M^++1, 100).
9	3376, 3317 (NH_2), 3195 (NH).	2.57 (s, 3H, SCH_3), 4.57 (br s, 2H, NH_2, D_2O-exchangeable), 7.85 (d, 2H, Ph), 8.28 (d, 2H, Ph), 9.25 (s, 1H, CH-pyrimidine), 9.65 (s, 1H, NH, D_2O-exchangeable),.	14.4 (1C, SCH_3), 113.4 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 142.7 (1C, C_3), 146.4 (1C, Ph), 146.5 (1C, Ph), 149.5 (1C, C_9), 154.4 (1C, CH-pyrimidine), 167.9 (1C, C-$NHNH_2$).	317 (M^+, 32), 287 (96), 122 (100).
10	1615 (C=N).	2.57 (s, 3H, SCH_3), 7.85 (d, 2H, Ph), 8.21 (d, 2H, Ph), 9.48 (s, 1H, CH-pyrimidine).		321 (M^+, 100).
11	1622 (C=N).	2.54 (s, 3H, SCH_3), 7.85 (d, 2H, Ph), 8.15 (d, 2H, Ph), 8.44 (s, 1H, CH-triazole), 9.29 (s, 1H, CH-pyrimidine).	15.2 (1C, SCH_3), 113.5 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 143.6 (1C, C_3), 143.9 (1C, CH-pyrimidine), 146.1 (1C, Ph), 146.2 (1C, Ph), 147.6 (1C, C-pyrimidine), 149.5 (1C, C_9), 151.8 (1C, CH-triazole).	327 (M^+, 12), 69 (100).

TABLE 2. (Continued).

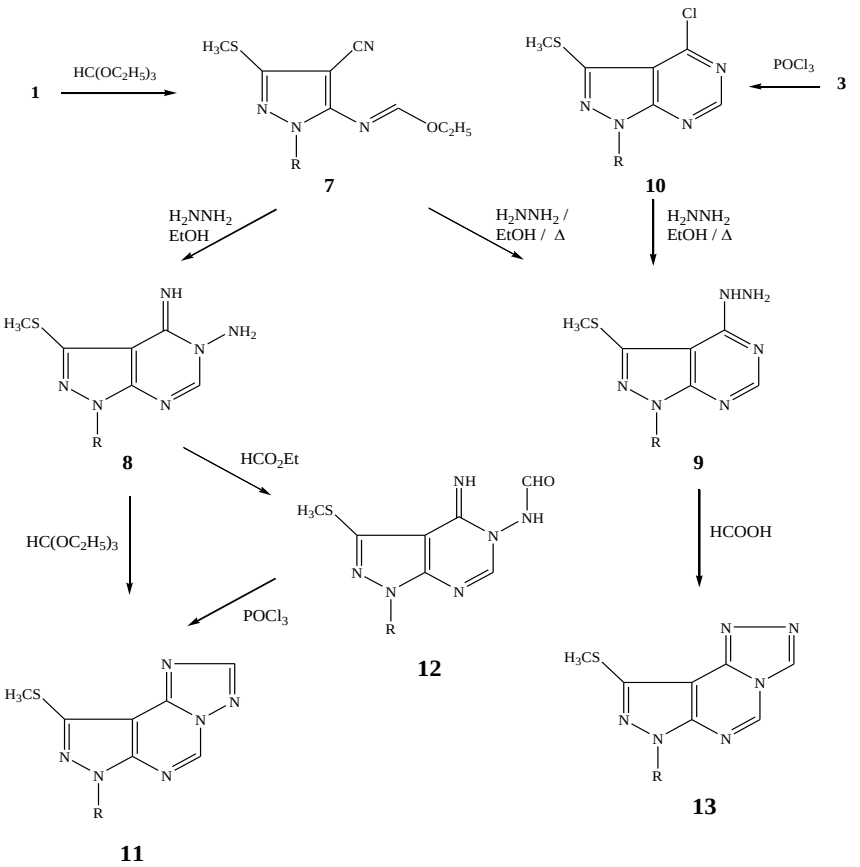
Compd. No.	IR ν (cm-1)	1H NMR δ (ppm)	13C NMR δ (ppm)	MS m/z (I, %)
12	3221 (NH) 1628 (C=N).	2.55 (s, 3H, SCH_3), 7.77 (d, 2H, Ph), 7.85 (s, 1H, =NH, D_2O-exchangeable), 8.17 (d, 2H, Ph), 8.43 (s, 1H, CH-pyrimidine), 9.1 (s, 1H, NH, D_2O-exchangeable).		346 (M^++1, 6), 236 (100).
13	1635 (C=N).	2.55 (s, 3H, SCH_3), 7.87 (d, 2H, Ph), 8.14 (d, 2H, Ph), 8.45 (s, 1H, CH-triazole), 9.30 (s, 1H, CH-pyrimidine).	15.1 (1C, SCH_3), 113.5 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 143.2 (1C, CH-triazole), 143.6 (1C, C_3), 144.6 (1C, CH-pyrimidine), 146.1 (1C, Ph), 146.2 (1C, Ph), 149.5 (1C, C_9), 151.7 (CH-triazole).	327 (M^+, 100).
15	1639 (C=N).	δ 2.57 (s, 3H, SCH_3), 7.87 (d, 2H, Ph), 8.16 (d, 2H, pyridine-H_3,H_5), 8.57 (d, 2H, Ph), 8.82 (d, 2H, pyridine-H_2,H_6), 9.41 (s, 1H, CH-pyrimidine).	15.1 (1C, SCH_3), 110.2 (1C, C_4), 125.1 (2C, Ph), 125.3 (2C, Ph), 120.1 (2C, C_3,C_5-pyridine), 133.6 (1C, C_4-pyridine), 142.5 (1C, C_3), 143.8 (1C, CH-pyrimidine), 146.7 (1C, Ph), 147.8 (1C, Ph), 149.4 (1C, C-pyrimidine), 149.5 (1C, C_9), 150.2 (2C, C_2,C_6-pyridine), 162.4 (1C, C-triazole).	405 (M^++1, 7), 359 (85), 209 (58), 78 (100).
17	3145 (NH).	2.54 (s, 3H, SCH_3), 7.47-8.53 (m, 9H, Ph), 8.58 (s, 1H, NH, D_2O-exchangeable), 9.23 (s, 1H, CH-pyrimidine).	15.1 (1C, SCH_3), 110 (1C, C_4), 119.4 (2C, Ph), 120.1 (1C, Ph), 125.1 (2C, Ph), 125.3 (2C, Ph), 130.4 (2C, Ph), 142.5 (1C, C_3), 143.8 (1C, CH-pyrimidine), 145 (1C, Ph), 146.1 (1C, Ph), 146.2 (1C, Ph), 149.4 (1C, C-pyrimidine), 149.5 (1C, C_9), 158.3 (1C, C-triazole).	418 (M^+, 3), 331 (100).



Scheme 1 .

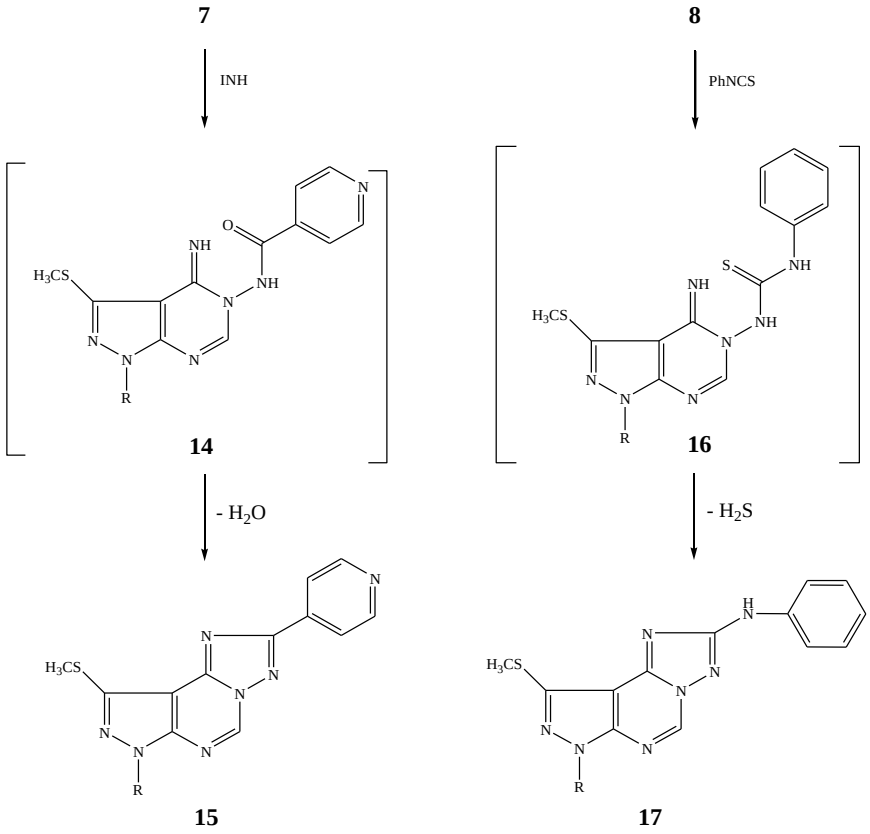
The reaction of the aminocyanopyrazole 1 with a mixture of triethylorthoformate and acetic anhydride afforded 5-ethoxymethylidene-amino-3-methylthio-1-(4`-nitrophenyl)-pyrazole-4-carbonitrile (7) . The latter underwent cyclocondensation reaction by the action of hydrazine hydrate, when its ethanolic solution was stirred at room temperature to give the aminoimino derivative 8. Meanwhile, 7 underwent cyclocondensation reaction by the action of hydrazine hydrate in refluxed ethanol to give the hydrazino derivative 9, in a 95% yield. This may proceeded *via* Dimroth-like rearrangement, in which thermal rearrangement of the aminoimino derivative 8 to its thermodynamically more stable hydrazine isomer 9 took place, in accordance with previous investigations[18]. The ^{1}H NMR spectrum of 8 showed the pyrimidine proton at δ= 8.23 ppm and that of 9 is more deshielded at 9.25 ppm, which could be due to long range effect of the hydrazino group rather than that of the imine one.

The structure of 9 was proved by its independent synthesis, by chlorination of the pyrazolo [3,4-*d*]pyrimidin-4-one derivative 3 using phosphorous oxychloride to give the pyrazolo[3,4-*d*]pyrimidin-4-chloro derivative 10. Product 10 underwent nucleophilic displacement of chlorine atom when subjected to the action of hydrazine hydrate to give the corresponding hydrazino derivative 9 (Method B), as identified by its melting point and TLC behavior (Scheme 2). As described in Scheme 2, reaction of 8 with triethyl orthoformate afforded the corresponding 9-methylthio-7-(4`-nitrophenyl)pyrazolo [4,3-*e*] [1,2,4] triazolo [1,5-*c*] pyrimidine 11. Product 11 was also obtained by the reaction of 8 with ethyl formate, which gave the formylamino intermediate 12 which underwent ring closure in refluxing phosphoryl chloride to give 11, in accordance with an other investigation[19]. Meanwhile, the reaction of the hydrazino derivative 9 with refluxed formic acid afforded the unsubstituted isomer 9-methylthio-7-(4`-nitrophenyl) -pyrazolo [4,3-*e*] [1,2,4] triazolo [4,3-*c*] pyrimidine (13) in 65% yield.



Scheme 2

As shown in Scheme 3, the ethoxymethylidene-amino derivative 7 was reacted with the less nucleophilic isonicotinic acid hydrazide (INH) in refluxed dioxane, in the presence of triethylamine, to afford pyrazolo[4,3-*e*] [1,2,4]-triazolo[1,5-*c*]pyrimidine derivative 15. The formation of 15 is predominated rather than the hydrazone derivative 14, which may be formed as an intermediate. The structure of 15 was based on analogy[20], correct analytical and spectral data. The EI mass spectrum of 15 identified the molecular ion peak at 404 (M^+, 100%), which is corresponding to the exact mass of its molecular formula $C_{18}H_{12}N_8O_2S$. Its ^1H NMR showed the pyrimidine proton as a singlet at δ 9.41 ppm and its IR spectrum lacks any band corresponding to primary or secondary amines.



Scheme 3.

On the other hand, the amino-imino derivative 8 was treated with phenylisothicyanate in refluxing pyridine to afford the 2-phenylamino derivative 17. This could be preceded *via* the thiourea intermediate 16 followed by dehydrosulfurization. The EI mass spectrum and elemental analysis showed

that 17 had the same formula, $C_{19}H_{14}N_8O_2S$. Its ^{1}H NMR showed the pyrimidine proton as a singlet at δ 9.23 ppm and the presence of one exchangeable proton at δ 8.58 ppm assigned for one NH proton. The ^{13}C-NMR spectrum of the same compound exhibited the characteristic carbons at δ 15.1, 110, 119.4, 120.1, 125.1, 125.3, 130.4, 142.5, 143.8, 145, 146.1, 146.2, 149.4, 149.5, 158.3 ppm.

Pharmacology

Random screening of compounds and reference drug, Diclophenac®, was performed at two dose levels 2.5 and 5 mg kg^{-1} p.o. Compounds 17, 11, 13 and 5 showed potent anti-inflammatory activities with potent prostaglandin inhibition at the two dose levels tested. For these compounds, a similar activity profile was realized for the inhibition of plasma prostaglandine (PGE2) (Table 3).

The tested compounds of this series showed LD_{50} values above 1100 mg kg^{-1} p.o., except 2, 9, 15 and 17 with maximum in compound 7 (2457.98 mg kg^{-1} p.o). The ulcerogenic activity (UD_{50} mg kg^{-1} i.p.), showed that compounds 3, 7, 8, 9, 10 and 17 are safer than Diclophenac®, due to high ulcerogenic causing doses that were above 200 mg kg^{-1} i.p.

Conclusion

A series of pyrazolo [3,4-*d*] pyrimidine and pyrazolo [3,4-*e*] [1,2,4] triazolo [1,5-*c*] pyrimidine derivatives were newly synthesized using aminopyrazole carbonitrile 1 as a starting material and compounds 14 and 16 are intermediates. The obtained pharmacological screening results of the products showed that compounds 17, 11, 13 and 5 have better anti-inflammatory activities comparable to the reference drug Diclophenac® and high selectivity against COX-2. All tested products manifested highly LD_{50} values, so they are considered to be of high safety margins.

Experimental

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, UK) and are uncorrected. Microanalyses were performed with all final compounds on an Elementar-Vario EL (Elementar-Vario EL, Germany) (Micro-analytical Unit, Central Services Laboratory, National Research Centre, Cairo, Egypt). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, USA). ^{1}H-NMR spectra were run at 300 MHz in DMSO-d_6 as solvent using tetramethylsilane as internal standard. Mass spectra were recorded on a Shimadzu GCMS-QP 1000EX (EI, 70 eV) (Shimadzu, Japan) and Hewlett-Packard (EI, 70 eV) (Hewlett-Packard, USA). IR spectra were obtained with a Brucker-Vector 22 (Bruker Rhein-Stetten, Germany). All the reactions were monitored using thin layer chromatography (TLC) using silica gel aluminum sheets $60F_{254}$ (Merck).

TABLE 3. Anti-inflammatory, ulcerogenic activity and acute toxicity data of the prepared compounds 2-13, 15 and 17 .

Compd. No.	Acute toxicity (ALD_{50} mg Kg^{-1} p.o.)	Ulcerogenic activity (UD_{50} mg Kg^{-1} i.p.)	Anti-inflammatory activity		
			Dose mg kg^{-1} p.o.	% inhibition of oedema	% inhibition of plasma PGE_2
2	1079.98	191.80	2.5	00.00	00.00
			5	46.75	58.24
3	1422.99	228.90	2.5	00.00	00.00
			5	44.82	46.98
4	1513.67	198.70	2.5	68.06	72.89
			5	71.44	75.09
5	1166.98	195.90	2.5	80.55	90.98
			5	89.35	96.89
6	1833.44	144.90	2.5	73.38	71.07
			5	79.24	82.63
7	2457.98	211.09	2.5	00.00	00.00
			5	36.60	49.62
8	1213.55	201.16	2.5	47.50	58.68
			5	50.62	60.07
9	998.98	234.00	2.5	62.78	54.66
			5	64.88	69.79
10	1805.86	208.60	2.5	00.00	00.00
			5	38.42	46.54
11	1613.89	113.20	2.5	85.32	83.98
			5	91.06	90.00
12	1118.42	164.80	2.5	48.60	57.76
			5	51.55	63.00
13	1970.48	188.40	2.5	82.98	77.98
			5	87.98	86.00
15	987.67	199.40	2.5	67.98	65.15
			5	77.98	71.32
17	1079.14	223.89	2.5	94.20	85.31
			5	95.88	92.09
Diclofenac potassium	2345.87	66.70	2.5	81.09	75.89
			5	85.44	84.87

5-Amino-3- methylthio-1-(4`-nitrophenyl)pyrazole-4-carbonitrile (1)

Sodium hydride (50%) (9.6 g, 200 mmol) was added to DMSO (100 ml) during external cooling (ice-bath) and vigorous stirring over a period of 15 min. Malononitrile (13.2 g, 200 mmol) was then added and the reaction mixture was stirred for 1hr. Carbon disulfide (20 ml, excess) was added at the same temperature over 30 min (color became yellow), then stirred at room temperature for 45 min. The reaction mixture was cooled in an ice-bath and methyl iodide (14 ml, 200 mmol) was drop-wisely added (color became red), then stirring was continued for 1hr at room temperature. The reaction mixture was poured onto water and kept overnight in a refrigerator and the obtained brownish precipitate was collected by filtration, dried and used without further purification.

To a solution of 1,1`-dicyano-2,2`-dithiomethylethylene (17 g; 100 mol) and piperidine (3 ml) in dioxane (250 ml), *p*-nitrophenylhydrazine (15.3 g; 100 mmol) was added in portions, during vigorous stirring, over 1hr. The stirred mixture was left at room temperature overnight, and the formed solid was separated by filtration, crystallized from dioxane (decolorized with charcoal) to afford 1.

3-(Methylthio)-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2)

A solution of 1 (1.1 g; 4 mmol) in formamide (10 ml) and acetic anhydride (5 drops) was heated on sand bath at 150^{o}C for 10hr. The solvent was distilled off in vacuum, and the oily dark residue was triturated with water and left at room temperature. The formed solid was filtered off, dried and crystallized to give 2.

3-(Methylthio)-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (*3)*

A mixture of 1 (1.1 g; 4 mmol) and formic acid (15 ml, 85%) was refluxed for 5hr, cooled, and poured onto ice/water mixture. The formed white precipitate was separated by filtration, washed thoroughly with water, dried and crystallized to give 3.

3-(Methylthio)-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione (4)

A solution of 1 (1.1 g; 4 mmol) in a mixture of ethyl orthoformate (15 ml) and acetic anhydride (1 ml) was refluxed for 3hr. Excess solvent was distilled off in vacuum; the oily residue was dissolved in ethanolic NaSH solution (25 ml, 1*M*), refluxed for 6hr and left overnight. The reaction mixture was concentrated to its ½ volume, treated with cold water and acidified with acetic acid (pH = 2). The separated solid was collected by filtration to give 4.

4-Imino -3-(methylthio) -1- (4-nitrophenyl) pyrazolo [3,4-d] [1,3] thiazine-6 (1H,4H,7H)-thione (5)

A solution of 1 (1.1 g; 4 mmol) in dry pyridine (5 ml) and carbon disulfide (15 ml) was refluxed on a water bath at 80^{o}C for 10 hr and left at room temperature overnight. The excess solvent was distilled off under reduced

pressure, the residue was triturated with water and ethanol and the formed solid was separated by filtration to afford 5.

3-(Methylthio) -1- (4-nitrophenyl)-1H-pyrazolo [3,4-d] pyrimidine-4,6(5H,7H)-dithione (6)

Method A

To a solution of 1 (1.1 g; 4 mmol) in 10% alcoholic potassium hydroxide (12 ml), carbon disulfide (12 ml) was added. The reaction mixture was refluxed for 3hr, cooled, poured onto ice/water mixture and neutralized with 1*N* hydrochloric acid. The formed solid was filtered off, washed with water and dried to give 6.

Method B

A solution of 5 (0.35 g, 1 mmol) in 10% alcoholic potassium hydroxide (5 ml) was refluxed for 1hr. The reaction mixture was cooled, poured onto ice/water mixture and neutralized with 1*N* hydrochloric acid. The formed solid was filtered off, washed with water and dried to give 6 as identified by its melting point, mixed melting point and chromatographic behavior in comparison with an authentic sample from method A.

5-Ethoxymethylideneamino -3- methylthio-1- (4`-nitrophenyl)pyrazole -4 carbonitrile (7)

A mixture of 1 (41.25 g; 150 mmol), triethyl orthoformate (25 ml; 150 mmol) and acetic anhydride (25 ml) was refluxed for 5hr. The solvent was removed under reduced pressure and the residue was treated with water. The obtained solid was filtered off, dried and crystallized from ethanol to give 7.

3-Amino-4-imino-7-(4`-nitrophenyl)-5-methylthiopyrazolo[3,4-d]pyrimidine (8)

To a cold suspension (5-10^{o}C) of **7** (33.1 g; 100 mmol) in absolute ethanol (500 ml), hydrazine hydrate (30 ml, 85%) in ethanol (100 ml) was added over 1hr while vigorous stirring, at the same temperature. Then, the reaction mixture was stirred at room temperature for 3hr, and the obtained solid was filtered off, washed with aqueous ethanol, dried to afford 8.

1-[3-methylthio-1-(4`-nitrophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-yl] hydrazine (*9)*

Method A

A mixture of 7 (3.31 g, 10 mmol) and hydrazine hydrate (5 ml, excess) in ethanol (50 ml) was heated under reflux for 3hr. The formed yellowish white precipitate was filtered off dried and re-crystallized to afford 9.

Method B

A suspension of 3 (2.12 g, 7 mmol) in phosphorous oxychloride (15 ml) was refluxed for 3hr. The reaction mixture was cooled, poured onto ice/water mixture and the greenish yellow solid so formed was separated by filtration and crystallized from ethanol to give 4-chloro-3-methylthio-1-(4`-nitrophenyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (10).

A mixture of 10 (1.6 g, 5 mmol) and hydrazine hydrate (2 ml, excess) in ethanol (25 ml) was heated under reflux for 3hr. The formed yellowish white precipitate was filtered off and dried, re-crystallized from DMF to afford 9 as identified by its melting point, mixed melting point and chromatographic behavior in comparison with an authentic sample from method A.

9-Methylthio-7-(4`-nitrophenyl)-pyrazolo [4,3-e] [1,2,4] triazolo[1,5-c] pyrimidine (11)

Method A

A mixture of 8 (0.95 g, 3 mmol) and triethyl orthoformate (0.5 ml, 33 mmol) in dioxane (75 ml) was refluxed for 8hr. On cooling, the formed solid was separated by filtration and crystallized to afford 11 as a tan yellow powder.

Method B

A mixture of 8 (0.95 g, 3 mmol) and ethyl formate (5 ml, excess) in DMF (5 ml) was refluxed for 5hr. After cooling, the formed solid product was filtered off, washed with water, dried and crystallized to afford 3-formylmino-4-imino-7-(4`-nitrophenyl)-5-methylthiopyrazolo[3,4-*d*] pyrimidine (12) as white crystals.

A mixture of 12 (0.35 g, 1 mmol) in $POCl_3$ (5 ml) was heated under reflux for 3hr. After cooling, the reaction mixture was poured onto ice/water mixture and neutralized with ammonia solution. The formed solid was filtered off, dried and crystallized to afford 11, as buff crystals.

9-Methylthio-7-(4`-nitrophenyl)-pyrazolo[4,3-e] [1,2,4]triazolo[4,3-c] pyrimidine (13)

A mixture of 9 (1.59 g; 5 mmol) and formic acid (25 ml, 85%) was refluxed for 5hr, cooled, and poured onto ice/water mixture. The formed greenish yellow precipitate was separated by filtration, washed thoroughly with water, dried and crystallized to give 13.

9-Methylthio-7-(4`-nitrophenyl)-2-(4``-pyridyl) pyrazolo[3,4-e] [1,2,4] triazolo-[1,5-c]pyrimidine (15)

A mixture of 7 (0.99 g, 3 mmol), isonicotinic acid hydrazide (0.41 g, 3 mmol) and TEA (2 ml) in dioxane (25 ml) was refluxed for 6hr. After cooling, the obtained solid was filtered off, and crystallized from DMF to afford 15 as a yellow powder.

9-Methylthio-7-(4`-nitrophenyl)-2-phenylpyrazolo [3,4-e][1,2,4] triazolo-[1,5-c] - pyrimidine (17)

A suspension of 8 (0.95 g, 3 mmol) and phenylisothiocyanate (0.4 g ≈ 0.36 ml, 3 mmol) in pyridine (12 ml) was heated under reflux for 5hr. After cooling the reaction mixture was poured onto ice/water and neutralized with 1*N* HCl. The formed solid was filtered off, washed with water, dried and crystallized to afford 17.

Pharmacology

Animals

Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals (National Academy of Science of Egypt) and were approved by the Animals Studies Committee at Washington University. Adult male albino rats, weighing 150–180 g, fasted for 12 hr and used for determining the anti-inflammatory activity and LD_{50}.

Evaluation of anti-inflammatory activity

The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of Winter *et al.*[21, 22]. Groups of rats, each of 8 animals were orally dosed with the test compounds at a dose level of 2.5 and 5 mg/kg an hour before carrageenan challenge. Foot paw edema was induced by sub planter injection of 0.05 ml of 1% suspension of carrageenin in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animal was decapitated, blood was collected and the paws were rapidly excised.

The average weight of edema was estimated for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated; then percentage protection against edema was estimated (Table 3).

Diclophenac® (2.5 and 5 mg/kg) was employed as standard reference against which the test compounds were compared.

Estimation of plasma prostaglandin E_2 (PGE_2)

Heparinized blood samples were collected from rats (n= 8), plasma was separated by centrifugation at 12 000 x g for 2 min at 4°C and immediately stored frozen 20°C until use.

The designs correlate-EIA prostaglandin in E_2 (PGE_2) kit is a competitive immune assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature the excess reagents were washed away and the substrate was added. After a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro plate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples. The percentage inhibition of plasma PGE_2 for each compound was estimated[23, 24] (Table3).

Evaluation of acute toxicity study

The test compounds were administered orally at different dose levels in separate groups of animals. After 24 hr of drug administration percent mortality in each group was observed. From the data obtained, the lethal dose (LD_{50}) was calculated by the method of Smith[25].

Evaluation of ulcerogenic activity

Ulcerogenic activity was determined according to the method of Verma *et al*. In this method[26], adult albino rats, fasted 24 hr prior to the administration of drugs, were divided into groups of ten animals each.

Water was allowed ad libitum to the animals. The test compounds and standard drugs were given intraperitoneally and the animals sacrificed 8 hr after drugs treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) petechial and frank haemorrhage and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

References

1. **Kontogiorgis**, **C.A**. **and Hadjipavlou-Litina**, **D.J.**, Non steroidal anti-inflammatory and anti-allergy agents. *Current Med. Chem.*, **9**, 89 (2002).

2. **Abu Elmaati**, **T. M.**, Novel and facile synthesis of pyrazolo [3,4-*d*]-pyrimidines. *Z. Naturforsch*, **57**b, 1333 (2002).

3. **Bhuyan**, **P. J.**, **Borh, H. N. and Sandhu**, **J. S.**, Studies on uracils: A facile one-pot synthesis of pyrazolo[3,4-d]pyrimidines. *Tetrahedron Lett.* **43**, 895 (2002).

4. **Zacharie, B., Connolly, T. P., Rej, R., Attardo, G. and Penney, C. L.**, A short synthesis of 4-substituted 1-(hydroxyalkyl)-1H-pyrazolo[3,4-d]-pyrimidines. *Tetrahedron*, **52**(7), 2271 (1996).

5. **Hecht, S. M., Frye, R. B., Werner, D., Fukui, T. and Hawrelak, S. D.,** Synthesis and biological activity of pyrazolo[3,4-d]pyrimidine nucleosides and nucleotides related to tubercidin, toyocamycin, and sangivamycin. *Biochemistry*, **15**, 1005 (1976).

6. **Chebib, M. and Quinn, R. J.**, 1-Phenylpyrazolo[3,4-d]pyrimidines as adenosine antagonists: The effects of substituents at C4 and C6. *Bioorg. Med. Chem.* **5**, 311 (1997).

7. **Seela, F. and Becher, G.**, Synthesis of 7-halogenated 8-aza-7-deaza-2'-deoxyguanosines and related pyrazolo [3,4-*d*] pyrimidine 2'-deoxyribonucleosides. *Synthesis*, 207 (1998).

8. **Borhani, D. W., Calderwood, D. J, Friedman, M. M., Hirst, G. C., Leung A. K. W., McRae, B., Ratnofsky, S., Ritter, K. and Waegell, W.**, A-420983: A potent, orally active inhibitor of lck with efficacy in a model of transplant rejection. *Bioorg. Med. Chem. Lett*. **14**, 2613 (2004).

9. **Schenone, S., Bruno, O., Bondavalli, F., Ranise, A., Mosti, L., Menozzi, G., Fossa, P., Manetti, F., Morbidelli, L., Trincavelli, L., Martini, C. and Lucacchini, A.**, *Eur. J. Med. Chem.* **39**, 153 (2004).

10. **Cardoso,C. R., de Brito, F. C. F., da Silva, K. C. M., de Miranda, C. A. M., Fraga, C. A. M. and Barreiro, E. J.**, Design, synthesis and pharmacological evaluation of novel pyrazolo[3,4-b]thieno[2,3-d]pyridine acid derivatives: a new class of anti-inflammatory and anti-platelet agents. *Bioorg. Med. Chem. Lett.* **12**, 9 (2002).

11. **Swelam, S. A., Abd El-Salam, O. I. and Zaki, M. E. A**., Synthesis of some pyrazolo[3,4-d]pyrimidines and their triazole and tetrazole derivatives. *J. Serb. Chem. Soc.* **64**(11), 655 (1999).

12. **Abd El-Salam, O.I. and Abdullah, M.**, Synthesis, antibacterial, anti-inflammatory and antiallergic activities of 2,4-pyridinedicarbohydrazide and its related derivatives. *Al-Azhar Bull. Sci.* **19**(2), 65 (2008).

13. **Abd El-Salam, O. I., Abou El Ella, D. A., Ismail, N. S. M. and Abdullah, M.,** Synthesis, docking studies and anti-inflammatory activity of some 2-amino-5,6,7,8-tetrahydroquinoline-3-carbonitriles and their related compounds. *Pharmazie*, **64**(3), 147 (2009).

14. **Abd El-Salam, O. I., Fahmy, A. F. M., Mohamed, A. M., Elnaggar, D. H. and Hammam, A.G.**, Synthesis, anticancer and anti-inflammatory activities of 3,4-dihyro-7-nitrobenzo[b]oxepin-5(2H)-one and its related derivatives. *W. J. Chem.* **5**(1), 7 (2010).

15. **Tominaga, Y., Honkawa, Y., Hara, M. and Hosomi, A.**, Synthesis of pyrazolo[3,4-d]pyrimidine derivatives using ketene dithioacetals. *J. Heterocyclic Chem.* **27**, 775 (1990).

16. **Taylor, E. C. and McKillop, A.**, Advances in organic chemistry: Methods and results *(The Chemistry of Cyclic Enaminonitriles and o-Aminonitriles)*. John Wiley & Sons, Inc (Interscience Publishers), New York, vol. **7**, p. 237 (1970).

17. **Taylor, E. C. and McKillop, A. and Vromen, S.**, A simple, one-step synthesis of fused pyrimidinethiones. *Tetrahedron* **23**, 885 (1967).

18. **Nagamatsu, T. and Fujita, T.**, *Heterocycles*, **57**(4), 631 (2002).

19. **Baraldi, P. G., El-Kashef, H., Farghaly, A., Vanelle, P. and Fruttarolo, F.**, Synthesis of new pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines and related heterocycles.*Tetrahedron,* **60**, 5093 (2004).

20. **Al-Afaleq, E. I. and Abubshait, S. A.**, Heterocyclic *o*-aminonitriles: preparation of pyrazolo[3,4-*d*]-pyrimidines with modification of the substituents at the 1- position**.** *Molecules*. **6**, 621 (2001).

21. **Winter, C. A., Risely, E. A. and Nuss, G. W.**, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs . *Proc. Soc. Exp. Biol. Med.* **111**, 544 (1962).

22. **Winter, C. A., Risely, E. A. and Nuss, G. W.**, Anti-inflammatory and antipyretic activities of indomethacin, 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid . *J. Pharmacol. Exp. Ther.* **141**, 369 (1963).

23. **Herrmann, F., Lindemann, A., Gauss, J. and Mertelsmann, R.**, Cytokinestimulation of prostaglandin synthesis from endogenous and exogenous arachidonic acids in polymorphonuclear leukocytes involving activation and new synthesis of cyclooxygenase. *Eur. J. Immunol.* **20**, 2513 (1990).

24. **Weithmann, K. U., Jeske, S. and Schlotte, V.**, Effect of leflunomide on constitutive and inducible pathways of cellular eicosanoid generation. *Agents Actions*, **41**, 164 (1994).

25. **Smith, Q. E**., *Pharmacological Screening Tests Progress in Medicinal Chemistry*, Vol. 1, Butterworth, London (1960).

26. **Verma, M., Sinha, J. N., Gujrati, V. R., Bhalla, T. N., Bhargava, K. P. and Shanker, K.**, A new potent anti-inflammatory quinazolone. *Pharmacol. Res. Commun.* **13**, 967 (1981).

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التشييد والخصائص البنائية لبعض مشتقات بيرازولو[4،3-د] بيريميدين وتقييمها كمضادات للإلتهابات

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قسم الكيمياء العضوية التطبيقية ، *قسم الكيمياء الضوئية ، **قسم الكيمياء العضوية الفلزية واللافلزية – المركز القومى للبحوث – 12622 مصر و ***وحدة البحث – شركة هاى كير للأدوية – القاهرة – مصر .

تم تشييد سلسلة من مشتقات البيريميدين والترايازولوبيريميدين الجديدة باستخدام كربونيتريل الأمينوبيرازول 1 كمادة بادئة والمركبات 14 و 16 كمواد وسيطة. وتم تقييم الفاعلية البيولوجية للمركبات الناتجة كمواد مضادة للإلتهابات، واظهرت المركبات 17، 11، 13 و 5 على التوالي فاعلية اكبر مقارنة بعقار الديكلوفيناك كمادة قياسية. وأثبتت الدراسة أن جميع المركبات امنة الإستخدام حيث اظهرت جميعها سمية اقل من العقار المستخدم ولاتسبب القرح المعدية. وتم أثبات التركيب البنائى للمركبات المشيدة بتحاليل عناصرها وكذا الدراسات الطيفية المختلفة لها كالأشعة تحت الحمراء، والرنين المغناطيسى لعنصرى الهيدروجين والكربون بالأضافة لطيف الكتلة لها.