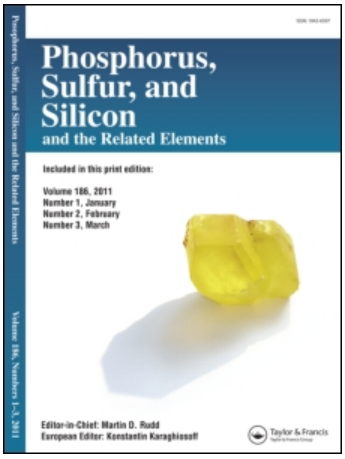

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Synthesis of Some Pyrazolo[3,4-d]pyrimidine Derivatives for Biological Evaluation

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*A novel β-enaminonitrile of 1-(6-p-tolyl-pyridazin-3-yl)-pyrazole derivative **2** was formed using (6-p-tolyl-pyridazin-3-yl)-hydrazine (**1**) and 2-ethoxymethylenemalononitrile. The β-enaminonitrile derivative **2** was in turn used as a precursor for the preparation of pyrazoles (**4, 6**), pyrazolo[3,4-d]-pyrimidines (**3, 7–12**) and pyrazolo[4,3-e][1,2,4]triazolo[4,3-c]pyrimidine (**13**). Also, N- and S-acyclic nucleosides **14** and **15** were prepared. Some of the prepared products showed potent antimicrobial activity.*

Keywords β-enaminonitrile; acyclic nucleosides; pyrazole; pyrazolo[3,4-*d*]pyrimidine; pyrazolo-[4,3-*e*]-[1,2,4]triazolo[4,3-*c*]pyrimidine

INTRODUCTION

Pyrazolo[3,4-*d*]pyrimidines have received a great attention due to their pharmacological activity,[1–4] such as allopurinol[5] (zyloric®), which is still the drug for treatment of hyperurecemia and gouty arthritic.[6–8] Various compounds with related structures also possess anti-tumor and anti-leukemia activities.[9,10] On the other hand, substituted pyridazines have received great interest, especially hydrazinopyridazine derivatives that were tested for their bactericidal, fungicidal,[11] and anti-hypertensive activity.[12] In continuation of our previous work on pyrazoles[13] and pyridazines,[13,14] we aimed to incorporate the pyridazine moiety into the 1-position of the pyrazolo[3,4-*d*]pyrimidine ring system to obtain a new compound which is expected to possess notable chemical and biological activities.

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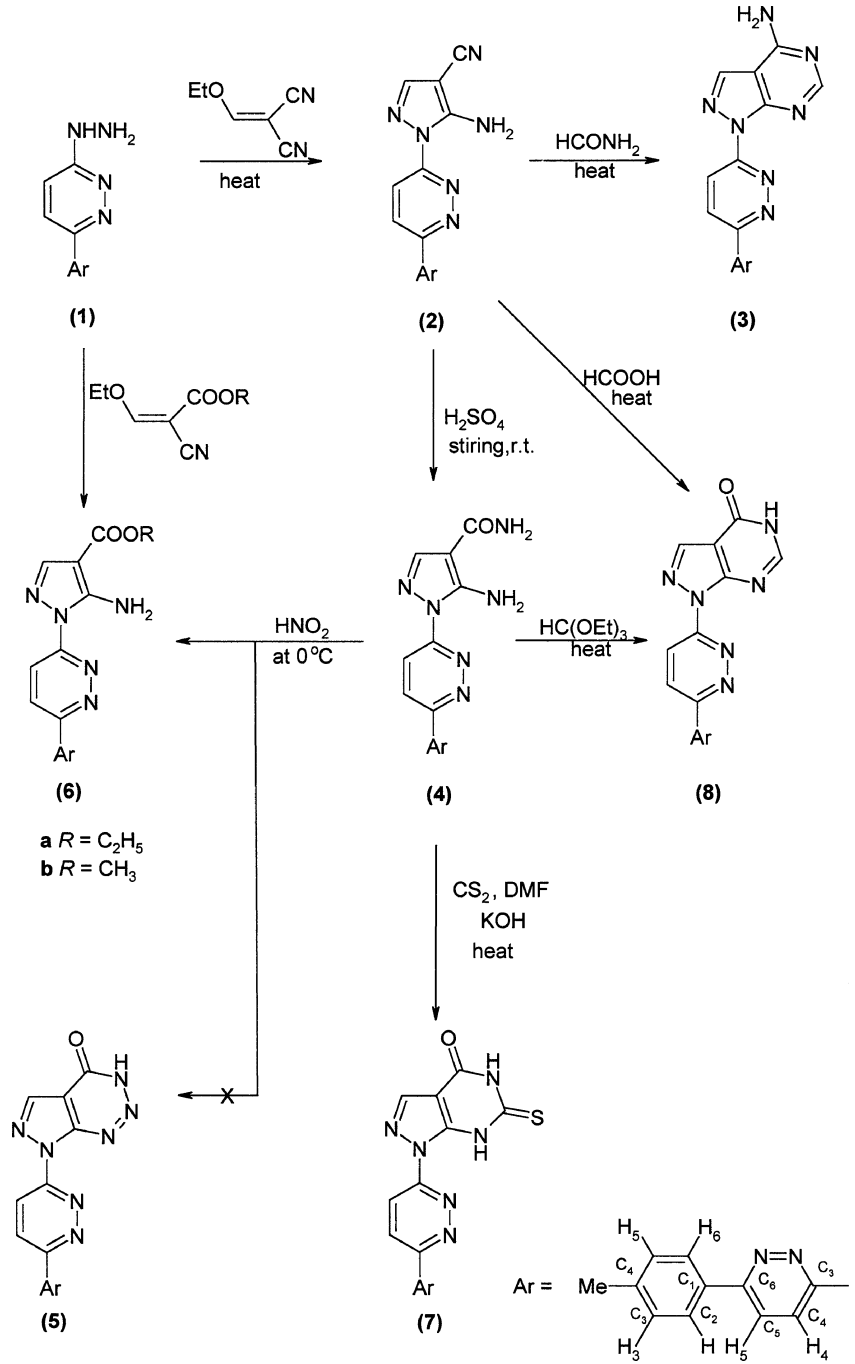
RESULTS AND DISCUSSION

This work is aimed at the synthesis of new compounds related to β-enaminonitriles of pyridazinyl pyrazole derivatives. In particular, 5-amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carbonitrile (**2**) was used as a key compound for this study and for further syntheses of other fused heterocyclic compounds. Thus, on refluxing a mixture of (6-*p*-tolylpyridazin-3-yl)-hydrazine (**1**)[15] and 2-ethoxymethylenemalononitrile in ethanol, compound **2** was obtained. The structure of compound **2** was confirmed with spectral data. Its IR spectrum showed bands at (ν, cm^{-1}): 3382, 3280 (NH_2) and 2220 (CN); ^1H NMR spectrum showed signals at (δ, ppm): 2.39 (s, 3H, *p*-CH_3-tolyl), 7.39 (d, 2H, $J = 7.8$ Hz, H3,H5-tolyl), 8.01 (s, 1H, H3-pyrazole), 8.06 (d, 2H, $J = 8.1$ Hz H2,H6-tolyl), 8.17 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.22 (brs, 2H, NH_2, exchangeable with D_2O), 8.43 (d, 1H, $J = 9.3$ Hz, H4-pyridazine); and MS gave the molecular ion peak as a base peak at $m/z = 276$. The synthesis of compound **3** was achieved by refluxing compound **2** with formamide. Its IR spectrum showed the absence of the cyano group and its ^1H NMR spectrum gave signals at (δ, ppm): 7.94 (brs, 2H, NH_2, exchangeable with D_2O) and 8.48–8.51 (m, 2H, H4-pyridazine and H6-pyrimidine).

On the other hand, compound **4** was obtained by stirring compound **2** with concentrated sulfuric acid at 0°C (Scheme 1). The spectral data of compound **4** assigned its structure (cf. Experimental). Many reports[16,17] stated that nitrozation of compounds analogous to **4** gave the corresponding triazinone derivatives; however, our attempts to cyclize compound **4** to **5** by stirring with nitrous acid gave the unexpected 5-amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid alkyl esters **6a,b** via formation of the diazonium chloride derivative, which on boiling in ethanol or methanol gave the corresponding esters **6a** ($R = C_2H_5$) or **6b** ($R = CH_3$), respectively (Scheme 1). The formation of compounds **6a,b** could be presumably explained by the formation of unstable diazonium salt on the amino of the amide moiety and not on the 5-amino group. Also, this could be established chemically when compound **1** was refluxed with 2-cyano-3-ethoxy-acrylic acid alkyl esters; it gave compounds **6a** and **6b**, respectively (Scheme 1).

When compound **4** was heated under reflux temperature with carbon disulphide in the presence of aqueous potassium hydroxide solution, it gave the corresponding 6-thioxo derivative **7**. Its ^1H NMR spectrum gave signals at (δ, ppm): 7.60 and 12.66: 2NH exchangeable with D_2O. On the other hand, when compound **4** was refluxed with triethyl orthoformate, it afforded 1-(6-*p*-tolyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (**8**), which could be obtained



SCHEME 1

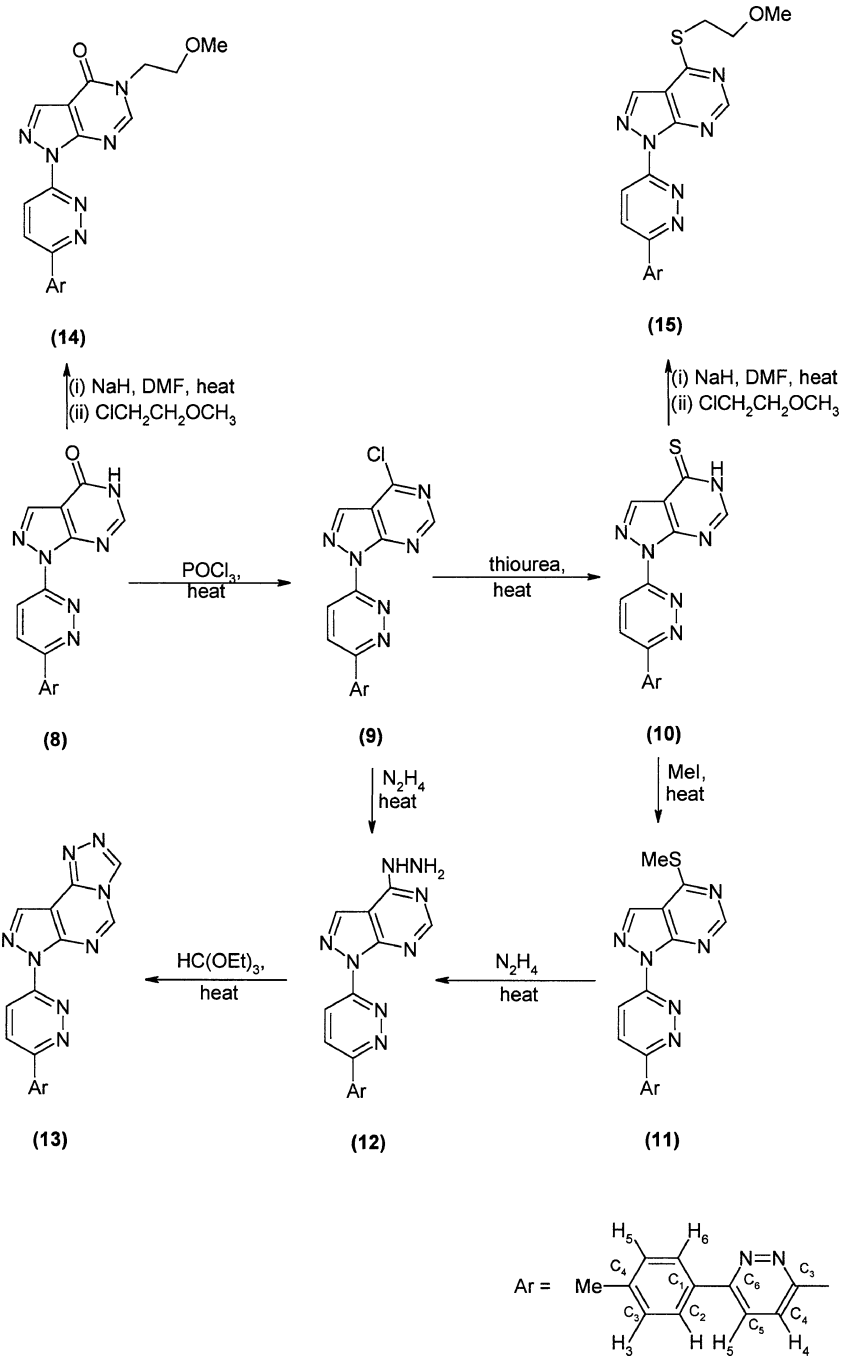
directly by refluxing compound **2** with formic acid. The formation of compound **8** from compound **2** presumably took place via hydrolysis of the cyano group to give compound **4**, which on prolonged heating, converted into compound **8**. The latter compound was converted into its corresponding 4-chloro derivative **9** by refluxing in phosphorus oxychloride, its MS, *m/z*: 324 (M^+, Cl^{37}, 38.41%), 322 (M^+, Cl^{35}, 100%).

Compound **10** was obtained from refluxing compound **9** with thiourea in dioxane according to a reported method.[18] Methylation of compound **10** with methyl iodide in the presence of potassium hydroxide solution afforded the corresponding 4-methylsulfanyl derivative **11**. Compound **12** was obtained either from compounds **9** or **11** by heating with hydrazine hydrate (Scheme 2). The ^{1}H NMR spectrum of compound **12** gave signals at (δ, ppm): 4.00 (brs, 1H, NH, exchangeable with D_2O) and at 7.47 (s, 2H, NH_2, exchangeable with D_2O); its MS gave the molecular ion peak as a base peak at $m/z = 318$.

When compound **12** was heated, in triethyl orthoformate it gave a product assigned to the structure of 7-(6-*p*-tolyl-pyridazin-3-yl)-7*H*-pyrazolo[4,3-*e*][1,2,4] triazolo[4,3-*c*]pyrimidine (**13**) (cf. Experimental Scheme 2).

Cyclic or acyclic pyrazolo[3,4-*d*]pyrimidine nucleosides that attached to the pyrimidine moiety are not known (to the best of our knowledge) in the literature. Actually, some acyclonucleosides were reported to be potent antiherpatic drugs with selective activity against Herpes Simplex Virus (HSV-1 and HSV-2) and Varicilla Zoster Virus (VZV), Human Cytomegalo Virus (HCMV), and Epestein-Barr Virus and also have shown high selectivity against Human Immunodeficiency Virus (HIV-1).[19–24] However, there is still a great need and interest to prepare more active acyclic pyrimidine nucleosides with less side effects than those observed for the already-known derivatives.

Therefore, when the sodium salts of compounds **8** and**10** (generated in situ) were treated with 2-chloroethyl methyl ether, they afforded the corresponding *N*-acyclic nucleoside: 5-(2-methoxy-ethyl)-1-(6-*p*-tolyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (**14**) and *S*-acyclic nucleoside: 4-(2-Methoxy-ethylsulfanyl)-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**15**), respectively (Scheme 2). The structure and sites of nucleosidation were elucidated with spectral data (cf. Experimental). The ^{13}C NMR spectra, as stated in similar compounds previously reported,[25] showed the presence of the C=O group in compound **14**, while that of compound **15** showed the absence of the C=S group.



SCHEME 2

BIOLOGICAL EVALUATION

The antimicrobial activity of some newly synthesized compounds **2**, **3**, **4**, **7**, **8**, **10**, **11**, and **15** were tested at concentration of 0.1 g/mL using dimethylformamide as a solvent.

Microorganisms' Species:

1. Bacteria
   a) Gram-negative bacteria, *Escherichia coli*
   b) Gram-positive bacteria, *Bacillus subtilis*
   Yeast: *Candida albicans*
2. Fungi: *Aspergillus Niger*

Medium

The cap-assay method containing (g/L): peptone 6.0, yeast extract 3.0, meat extract 1.5, glucose 1.0, and agar 20.0 were used. The medium was sterilized and divided while hot (50–60°C) in 15 mL portions among sterile petri dishes of 9 cm diameter.

One mL of the spor suspension of each microorganism was spread over the surface of the cold solid medium placed in the petri dish.

Method[26]

Each the tested compounds 0.5 g was dissolved in 5 mL of dimethylformamide. An amount of 0.1 mL of test solution was placed on watman paper disc of 9 mm diameter and the solvent was left to evaporate. These saturated discs were placed carefully on the surface of the inoculated solid medium; each petri dish contains at least 3 discs. The petri dishes were incubated at 5°C for 1 h to permit good diffusion and then transferred to an incubator of 85°C overnight and then examined. The results were recorded by measuring the inhibition zone diameters.

RESULTS

The antimicrobial activity of some newly synthesized compounds **2**, **3**, **4**, **7**, **8**, **11** and **13** were tested and the results were shown in Table 1. Among the compounds tested, it was noticed that compounds **4** and **7** showed more significant antibacterial and antifungal activity than some known drugs (standers). Compounds **2** and **3** demonstrated inhibitory activity against Gram positive bacteria and fungi. It was obvious that compounds **8** and **10** have only an inhibitory effect on fungi. On the other hand, compounds **11** and **15** showed activity against Gram positive bacteria and *Aspergillus Niger*.

TABLE I Test of Some Synthesized Compounds

	Inhibition zone (mm)			
	Microorganism			
	Bacteria			
Tested compounds and standers	Gram negative *Escherichia coli.*	Gram positive *Bacillus subtilis*	Fungi *Aspergillus niger*	Yeast *Candida albicans*
Streptomycin	+++	+++	+	+++
Erythromycin	–	+++	–	–
Ampicillin	++	–	–	–
Amoxicillin	++	–	–	–
Fusidic Acid	–	–	+++	+++
2	–	++	++	++
3	–	++	++	++
4	++	++	++	++
7	++	++	+++	++
8	–	–	++	++
10	–	–	++	++
11	–	++	++	–
15	–	++	++	–

+++ Highly sensitive (inhibition zone = 21–25 mm).
++ Fairly sensitive (inhibition zone = 16–20 mm).
+ Slightly sensitive (inhibition zone = 10–15 mm).
– No sensitivity.

Experimental

All melting points are uncorrected and were measured using an Electrothermal IA 9100 apparatus. Analytical data were performed by Vario El Mentar apparatus, organic microanalysis section, National Research Centre. Their results were found to be in agreement with the calculated values (±0.3). The IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer. ^{1}H & ^{13}C NMR spectra were determined on a Jeol 300 MHz in *DMSO*-d_6 and the chemical shifts were expressed in ppm relative to TMS as internal reference. Mass spectra were run at 70 eV on EI + Q1 MSLMR UPLR. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.2).

5-Amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carbonitrile (2)

To a solution of compound **1** (2.00 g, 0.01 mol) in ethanol (30 mL), 2-ethoxymethylene-malononitrile (1.22 g, 0.01 mole) was added. The

reaction mixture was heated at reflux temperature for 2 h. The precipitate that formed was filtered while hot, dried and recrystallized from dioxane to give **2** in 98% yield; m.p. 314–5°C; IR spectrum (KBr, ν, cm^{-1}): 3382, 3280 (NH_2) and 2220 (CN); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.39 (s, 3H, *p*-CH_3-tolyl), 7.39 (d, 2H, $J = 7.8$ Hz, H3,H5-tolyl), 8.01 (s, 1H, H3-pyrazole), 8.06 (d, 2H, $J = 8.1$ Hz H2,H6-tolyl), 8.17 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.22 (brs, 2H, NH_2, exchangeable with D_2O), 8.43 (d, 1H, $J = 9.3$ Hz, H4-pyridazine); MS, *m/z* (%): 276 (M^+, 100). Analysis for $C_{15}H_{12}N_6$ (276.30): required C, 65.21; H, 4.38; N, 30.42; found C, 65.24; H, 4.36; N, 30.40.

1-(6-*p*-Tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (3)

A mixture of compound **2** (2.76 g, 0.01 mol) and formamide (30 mL) was heated at 150°C for 3 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give **3** in 85% yield; m.p. 333–35°C. IR spectrum (KBr, ν, cm^{-1}): 3380, 3137 (NH_2), no absorption in the region 2260–2240 (CN, absent); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.41 (s, 3H, *p*-CH_3-tolyl), 7.40 (d, 2H, $J = 8.1$ Hz, H3, H5-tolyl), 7.94 (brs, 2H, NH_2, exchangeable with D_2O), 8.11 (d, 2H, $J = 8.1$ Hz H2, H6-tolyl), 8.34 (s, 1H, H3-pyrazole), 8.42 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.48–8.51 (m, 2H, H4-pyridazine and H6-pyrimidine); MS, *m/z* (%): 303 (M^+, 100). Analysis for $C_{16}H_{13}N_7$ (303.33): required C, 63.36; H, 4.32; N, 32.32; found C, 63.20; H, 4.34; N, 32.50.

5-Amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid amide (4)

To cold concentrated sulphuric acid (10 mL, 98%), compound **2** (2.76 g, 0.01 mol) was added portionwise with ice bath that was cooled and stirred. After 10 min, the ice bath was removed and the mixture was stirred for an additional 15 min. The resulting pale yellow solution was carefully poured onto ice chips, neutralized with ammonium hydroxide solution (5 mL, 25%), and the resulting precipitate was collected, washed with water, dried, and recrystallized from dioxane to give **4** in 81% yield; m.p. 276–8°C. IR spectrum (KBr, ν, cm^{-1}): 3423–3153 (2 NH_2), and 1662 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.40 (s, 3H, *p*-CH_3-tolyl), 7.10 (brs, 2H, NH_2, exchangeable with D_2O), 7.39 (d, 2H, $J = 7.8$ Hz, H3, H5-tolyl), 7.63 (s, 2H, NH_2, exchangeable with D_2O), 8.05–8.07 (m, 3H, H2, H6-tolyl and H3-pyrazole), 8.17 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.40 (d, 1H, $J = 9.3$ Hz, H4-pyridazine);

MS, m/z (%): 294 (M^+, 100). Analysis for $C_{15}H_{14}N_6O$ (294.32): required C, 61.22; H, 4.79; N, 28.55; found C, 61.12; H, 4.58; N, 28.75.

SYNTHESIS OF 6A AND 6B

Method A

To a suspended solution of compound **4** (2.94 g, 0.01 mol) in concentrated hydrochloric acid (30 mL, 34%) at 0–5°C, a solution of sodium nitrite (3.00 g) in water (5 mL) was added over 20 min. After 2 h of stirring at room temperature; the foamy mixture was filtered off. The resulting solid was dried and boiled in ethanol or methanol for 10 min. The precipitates that formed were filtered on hot and dried and recrystallized from an appropriate solvent to give **6a** and **6b**, respectively.

Method B

To a solution of compound **1** (2.00 g, 0.01 mol) in ethanol (30 mL), 0.01 mol of each of 2-cyano-3-ethoxy-acrylic acid ethyl ester (1.69 g, 0.01 mol) or 2-cyano-3-ethoxy-acrylic acid methyl ester (1.55 g, 0.01 mol) was added. The reaction mixtures were heated at reflux temperature for 2 h. The precipitates that formed were filtered while hot and dried and recrystallized from an appropriate solvent to give **6a** and **6b**, respectively. The obtained products were identified by m.p. and tlc in comparison with authentic samples from method A.

5-Amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid ethyl ester (6a)

Yield: (86%, dioxane); m.p. 218–20°C. IR spectrum (KBr, ν, cm^{-1}): 3448, 3330 (NH_2) and 1678 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 1.29 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.39 (s, 3H, *p*-CH_3-tolyl), 4.24 (q, 2H, $J = 7.2$ Hz, OCH_2CH_3), 7.37 (d, 2H, $J = 8.1$ Hz, H3,H5-tolyl), 7.71 (brs, 2H, NH_2, exchangeable with D_2O), 7.88 (s, 1H, H3-pyrazole), 8.06 (d, 2H, $J = 7.2$ Hz, H2, H6-tolyl), 8.19 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.42 (d, 1H, $J = 9.3$ Hz, H4-pyridazine); MS, *m/z* (%): 323 (M^+, 100). Analysis for $C_{17}H_{17}N_5O_2$ (323.36): required C, 63.15; H, 5.30; N, 21.66; found C, 63.28; H, 5.21; N, 21.59.

5-Amino-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid methyl ester (6b)

Yield: (97%, benzene); m.p. 180–2°C. IR spectrum (KBr, ν, cm^{-1}): 3416, 3305 (NH_2) and 1700 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.40

(s, 3H, *p*-CH_3-tolyl), 3.75 (s, 3H, OCH_3), 7.37 (d, 2H, $J = 8.1$ Hz, H3, H5-tolyl), 7.72 (brs, 2H, NH_2, exchangeable with D_2O), 7.89 (s, 1H, H3-pyrazole), 8.06 (d, 2H, $J = 7.2$ Hz, H2, H6-tolyl), 8.20 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.43 (d, 1H, $J = 9.3$ Hz, H4-pyridazine); MS, *m/z* (%): 309 (M^+, 100).

Analysis for $C_{16}H_{15}N_5O_2$ (309.33): required C, 62.13; H, 4.89; N, 22.64; found C, 62.13; H, 4.89; N, 22.64.

6-Thioxo-1-(6-*p*-tolyl-pyridazin-3-yl)-1,5,6,7-tetrahydro-pyrazolo[3,4-*d*]pyrimidin-4-one (7)

To a solution of compound **4** (2.94 g, 0.01 mol) in dimethylformamide (30 mL), 20% potassium hydroxide solution (potassium hydroxide 1.68 g, water 7 mL) and carbon disulfide (5 mL) were added. The reaction mixture was heated under reflux temperature for 15 h, cooled, poured into water, neutralized with hydrochloric acid (3 mL, 34%), filtered off, dried, and recrystallized from dimethylformamide to give **7** in 76% yield; m.p. 308–10°C. IR spectrum (KBr, ν, cm^{-1}): 3344 (NH), 3320 (NH); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.35 (s, 3 H, *p*-CH_3-tolyl), 7.38 (d, 2H, $J = 8.1$ Hz, H3, H5-tolyl), 7.60 (s, 1H, NH, exchangeable with D_2O), 8.09 (d, 2H, $J = 8.1$ Hz, H2, H6-tolyl), 8.21 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.36 (s, 1H, H3-pyrazole), 8.51 (d, 1H, $J = 9.6$ Hz, H4-pyridazine) and 12.66 (s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 336 (M^+, 73.97), 319 (10.37), 294 (100). Analysis for $C_{16}H_{12}N_6OS$ (336.38): required C, 57.13; H, 3.60; N, 24.98; S, 9.53; found C, 57.21; H, 3.52; N, 25.01; S, 9.44.

1-(6-*p*-Tolyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]-pyrimidin-4-one (8)

Method A

Compound **4** (2.94 g, 0.01 mol) was heated under reflux temperature in triethyl orthoformate (30 mL) for 10 h. The product, which separated on cooling, was filtered off, washed with ethanol, dried, and recrystallized from dimethylformamide to give **8** in 86% yield, m.p. 383–4°C.

Method B

Compound **2** (2.76 g, 0.01 mol) was heated under reflux temperature in formic acid (30 mL, 85%) for 5 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dimethylformamide to give **8** in 78% yield, which

was identical in all aspects with compound **8** obtained from method A, m.p. 383–4°C. IR spectrum (KBr, ν, cm^{-1}): 3102 (NH), and 1700 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.41 (s, 3H, *p*-CH_3-tolyl), 7.40 (d, 2H, $J = 7.8$ Hz, H3, H5-tolyl), 8.11 (d, 2H, $J = 7.5$ Hz, H2, H6-tolyl), 8.23 (s, 1H, H3-pyrazole), 8.30 (d, 1H, $J = 9.3$ Hz, H5-pyridazine), 8.45–8.48 (m, 2H, H4-pyridazine and H6-pyrimidine) and 12.51 (brs, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 304 (M^+, 100). Analysis for $C_{16}H_{12}N_6O$ (304.31): required C, 63.15; H, 3.97; N, 27.62; found C, 63.20; H, 4.05; N, 27.48.

4-Chloro-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (9)

Compound **8** (1.52 g, 0.005 mol) was heated in phosphorus oxychloride (20 mL) for 10 h. The solution was cooled and poured into ice water. The solid was filtered off, dried, and purified on silica gel using chloroform /n-hexane (1:1) as an eluent to give compound **9** in 55% yield, m.p. 274–6°C. IR spectrum (KBr, ν, cm^{-1}): no absorption in the region of (C=O); MS, *m/z* (%): 324 (M^+, Cl^{37}, 32.93), 322 (M^+, Cl^{35}, 100).

1-(6-*p*-Tolyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]-pyrimidine-4-thione (10)

Compound **9** (1.62 g, 0.005 mol) and (0.38 g, 0.005 mol) of thiourea in dioxane (40 mL) was heated at reflux for 4 h. The precipitate that formed was collected and dissolved in sodium hydroxide (20 mL, 10%). The mixture was filtered off and the filtrate was precipitated with hydrochloric acid (5 mL, 34%). The solid product was collected and recrystallized from dimethylformamide to give **10** in 75% yield; m.p. 352–4°C. IR spectrum (KBr, ν, cm^{-1}): 3025 (NH), and 1365 (C=S); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.40 (s, 3H, *p*-CH_3-tolyl), 7.40 (d, 2H, $J = 8.4$ Hz, H3, H5-tolyl), 8.11 (d, 2H, $J = 7.8$ Hz, H2, H6-tolyl), 8.27–8.34 (m, 2H, H3-pyrazole and H5-pyridazine), 8.48 (d, 1H, $J = 9.0$ Hz, H4-pyridazine), 8.57 (s, 1H, H6-pyrimidine) and 13.96 (s, 1H, NH, exchangeable with D_2O). MS, *m/z* (%): 320 (M^+, 100). Analysis for $C_{16}H_{12}N_6S$ (320.38): required C, 59.98; H, 3.78; N, 26.23; S, 10.01; found C, 60.11; H, 3.85; N, 26.10; S, 9.95.

4-Methylsulfanyl-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (11)

Compound **10** (1.60 g, 0.005 mol) in ethanol (20 mL), and sodium hydroxide solution (5 mL, 10%) were treated with methyl iodide (0.005 mol).

The reaction mixture was refluxed for 5 h, cooled, and poured into water. The separated solid was filtered off, dried, and recrystallized from dioxane to give **11** in 86% yield, m.p. 238–40°C. ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.39 (s, 3H, *p*-CH_3-tolyl), 2.75 (s, 3H, SCH_3), 7.38 (d, 2H, $J = 7.8$ Hz, H3, H5-tolyl), 8.09 (d, 2H, $J = 8.1$ Hz H2, H6-tolyl), 8.43–8.45 (m, 2H, H5, H4-pyridazine), 8.74 (s, 1H, H3-pyrazole), and 8.92 (s, 1H, H6-pyrimidine); MS, *m/z* (%): 334 (M^+, 100).

1-(6-*p*-Tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*-pyrimidin-4-yl]-hydrazine (12)

Method A

Compound **9** (1.62 g, 0.005 mol) was heated in hydrazine hydrate (20 mL, 98%) for 2 h, cooled, poured into water, neutralized with hydrochloric acid (2 mL, 34%), filtered off, dried, and purified with column chromatography (silica gel) using chloroform: methanol (9:1), as an eluent to give **12** in 57% yield, m.p. 344–6°C.

Method B

To a solution of compound **11** (1.67 g, 0.005 mol) in dioxane (30 mL), hydrazine hydrate (2 mL, 98%) was added and the mixture was heated at reflux for 4 h. The reaction mixture was cooled and poured into water and the separated solid was filtered off, dried, and recrystallized from dioxane to give **12** in 66% yield which was identical in all aspects with compound **12** obtained from method A, m.p. 344–6°C. IR spectrum (KBr, ν, cm^{-1}): 3425, 3387 (NH_2), and 3310 (NH); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.40 (s, 3H, *p*-CH_3-tolyl), 4.00 (brs, 1H, NH, exchangeable with D_2O), 7.39 (d, 2H, $J = 8.4$ Hz, H3, H5-tolyl), 7.47 (s, 2H, NH_2, exchangeable with D_2O), 8.04–8.09 (m, 3H, H2, H6-tolyl and H3-pyrazole), 8.21–8.28 (m, 2H, H5, H4-pyridazine), and 8.43 (s, 1H, H6-pyrimidine); MS, *m/z* (%): 318 (M^+, 100). Analysis for $C_{16}H_{14}N_8$ (318.34): required C, 60.37; H, 4.43; N, 35.20; found C, 60.20; H, 4.50; N, 35.29.

7-(6-*p*-Tolyl-pyridazin-3-yl)-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo-[4,3-c]pyrimidine (13)

Compound **12** (1.59 g, 0.005 mol) was heated under reflux temperature in 20 mL triethyl orthoformate for 15 h. The reaction mixture was filtered while hot and the filtrate was evaporated untill dry and the remaining solid was recrystallized from dioxane to give **13** in 56% yield,

m.p. 308–310°C. ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.42 (s, 3H, *p*-CH_3-tolyl), 7.42 (d, 2H, $J = 8.1$ Hz, H3, H5-tolyl), 8.15 (d, 2H, $J = 8.1$ Hz, H2, H6-tolyl), 8.39 (d, 1H, , $J = 9.0$ Hz, H5-pyridazine), 8.53 (d, 1H, $J = 9.0$ Hz, H4-pyridazine), 8.73 (s, 1H, H3-pyrazole), 8.93 (s, 1H, H8-pyrimidine), and 9.79 (s, 1H, H6-triazole); MS, *m/z* (%): 328 (M^+, 100).

SYNTHESIS OF 14 AND 15

General Procedure

A mixture of compound **8** (3.00 g, 0.01 mol) or **10** (3.20 g, 0.01 mol) and 50% oil-immersed sodium hydride (0.48 g, 0.02 mol) in dry dimethylformamide (50 mL) was stirred at 70°C for 1 h and then cooled to room temperature. 2-Chloroethyl methyl ether (0.95 g, 0.01 mol) was added and stirred at 90°C for 8 h. The mixture was evaporated until dry under reduced pressure and the solids were chromatographed on silica gel column with chloroform: methanol mixture (9:1) to give **14** or **15**.

5-(2-Methoxy-ethyl)-1-(6-*p*-tolyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (14)

Yield 77%; m.p. 269–70°C. IR spectrum (KBr, ν, cm^{-1}): 1691 (C=O); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.40 (s, 3H, *p*-CH_3-tolyl), 3.27–3.34 (m, 5 H, OCH_3 and CH_2O), 3.65 (t, 2 H, CH_2N), 7.39 (d, 2H, $J =$ 7.8 Hz, H3, H5-tolyl), 8.11 (d, 2H, $J =$ 7.8 Hz, H2, H6-tolyl), 8.44–8.47 (m, 2H, H5, H4-pyridazine), 8.75 (s, 1H, H3-pyrazole), and 8.92 (s, 1H, H6-pyrimidine); ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 21 (*p*-$\underline{C}H_3$-tolyl), 32 ($O\underline{C}H_3$), 58 ($\underline{C}H_2O$), 69 ($\underline{C}H_2N$), 108 (C3a), 137 (C3), 140 (C6), 159 (C7a), 159 (C=O), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 127, 130, and 152 (C4, C3, C2, and C1 tolyl). Analysis for $C_{19}H_{18}N_6O_2$(362.39): required C, 62.97; H, 5.01; N, 23.19; found C, 63.12; H, 4.90; N, 23.10.

4-(2-Methoxy-ethylsulfanyl)-1-(6-*p*-tolyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (15)

Yield 82%; m.p. 217–19°C; ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 2.40 (s, 3H, *p*-CH_3-tolyl), 3.27–3.34 (m, 5 H, OCH_3 and CH_2O), 3.65 (t, 2H, CH_2S), 7.39 (d, 2H, $J =$ 7.8 Hz, H3, H5-tolyl), 8.11 (d, 2H, $J =$ 7.8 Hz, H2, H6-tolyl), 8.40–8.50 (m, 2H, H5, H4-pyridazine), 8.75 (s, 1H, H3-pyrazole), and 8.92 (s, 1H, H6-pyrimidine); ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 22 (*p*-$\underline{C}H_3$-tolyl), 29 ($O\underline{C}H_3$), 59 ($\underline{C}H_2O$), 72 ($\underline{C}H_2S$), 133 (C3a), 136 (C3), 141 (C6), 159 (C7a), 164 (C4), 122, 126, 153, and 156

(C5, C4, C6, and C3 pyridazine), 114, 127, 130, and 152 (C4, C3, C2, and C1 tolyl)) ; MS, *m/z* (%): 378 (M^+, 8.35), 333 (17), 320 (100).

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