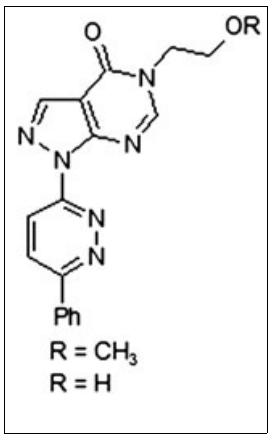
# Some New Pyrazole and Pyrazolopyrimidines: Synthesis and Antimicrobial Evaluation

Ahmed H. Shamroukh,[a,b]* Aymn E. Rashad,[a,c] Hatem S. Ali,[d] and Farouk M. E. Abdel-Megeid[a]

[a]Photochemistry Department, National Research Center, Dokki, Cairo, Egypt
[b]Chemistry Department, Faculty of Science, Hail University, Hail, Saudi Arabia
[c]Chemistry Department, Faculty of Science and Humanities, Shaqra University, Shaqra, Huraymila, Saudi Arabia
[d]Food Science and Nutrition Department, Faculty of Food Science and Agriculture, King Saud University, Riyadh, Saudi Arabia
*E-mail: ahshamroukh@yahoo.com

Received September 15, 2011
DOI 10.1002/jhet.1550
Published online 00 Month 2013 in Wiley Online Library (wileyonlinelibrary.com).




Novel $\beta$-enaminonitrile of 1-(6-phenyl-pyridazin-3-yl)-pyrazole derivative **2** was formed using (6-phenyl-pyridazin-3-yl)-hydrazine (**1**) and 2-ethoxymethylene-malononitrile. The $\beta$-enaminonitrile derivative **2** was in turn used as precursors for the preparation of 1-(6-phenyl-pyridazin-3-yl)-pyrazoles (**3, 9, 11**), 1-(6-phenyl -pyridazin-3-yl)-pyrazolo[3,4-*d*]pyrimidines (**4–8**, **13–16**) and some of their corresponding N-acyclic nucleosides (**17**, **18**). All synthesized compounds were tested for their antimicrobial evaluation, and compounds **3**, **9**, **17**, and **18** showed more significant activity than the other tested compounds and some known drugs (standers).

*J. Heterocyclic Chem.*, **00,** 00 (2013).

## INTRODUCTION

The chemistry of pyrazolopyrimidine derivatives has attracted the attention of numerous researchers over many years because of their important biological activities [1–3]. The structural similarity of pyrazolo[3,4-*d*]pyrimidines with purines [4] has made them a prime target for scientific research; and in this context, several reports dealing with the synthesis of these fused heterocyclic compounds have been appeared in the literature [4–7]. Pyrazolopyrimidines exhibited various biological activities such as antimicrobial [6] and antitumor [8]. In addition, a literature survey revealed that pyrazolopyrimidines have been received much attention on account of their neuroleptic [9], antihypertensive [10,11], antileishmanial [12], analgesic [13], and antimicrobial [14] activities. Also, the chemistry of pyridazine derivatives has received great attention, especially hydrazinopyridazine derivatives that were prepared and tested for their biological activity [15]. In continuation of our previous work on pyridazines and pyrazoles [15–17], we aimed to incorporate the pyridazine moiety into 1-position of the pyrazolo[4,3-*d*] pyrimidine ring system to obtain a new heterocyclic ring system that is expected to possess notable chemical and biological activities.

## RESULTS AND DISCUSSION

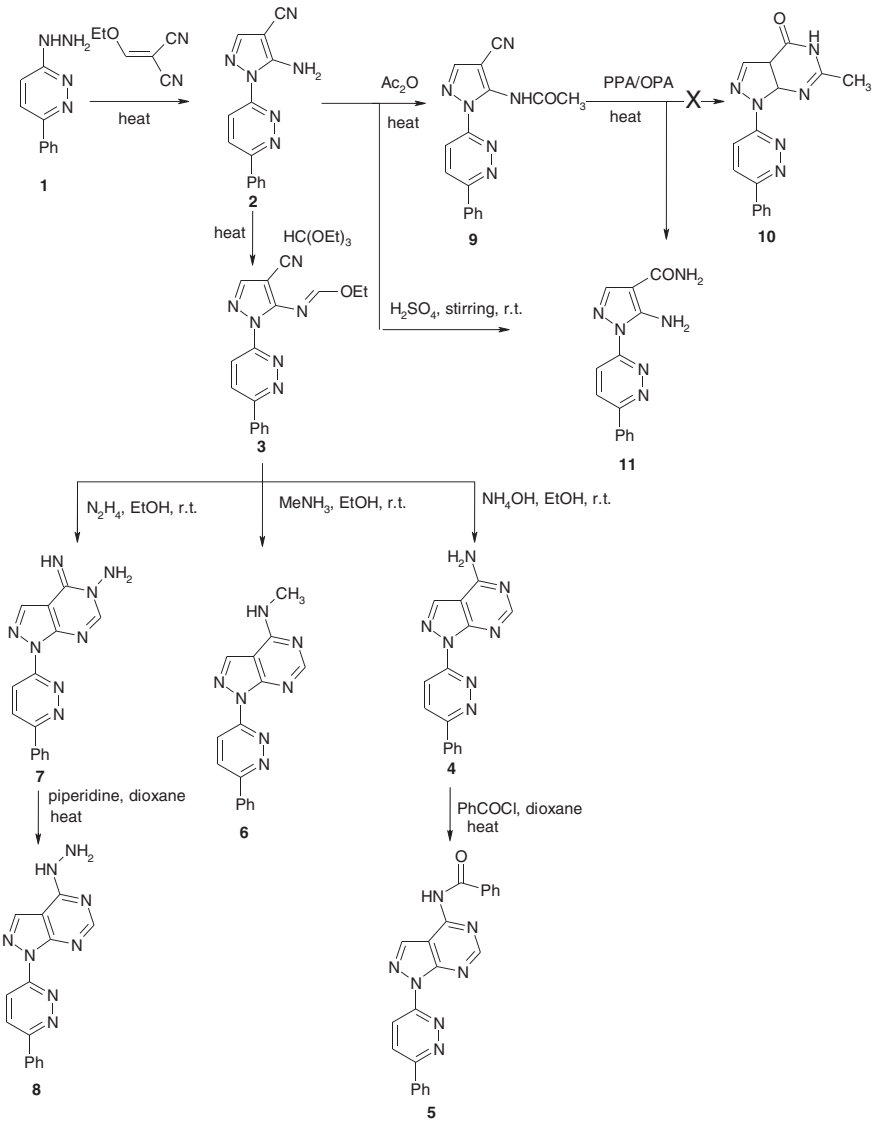
This work is aimed at the synthesis of new compounds related to $\beta$-enaminonitriles of pyridazinyl pyrazole derivatives. In particular, 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carbonitrile (**2**) was used as key compound for this study and for further syntheses of other fused heterocyclic compounds. Compound **2** was prepared by refluxing a mixture of 6-phenylpyridazin-3-ylhydrazine (**1**) [18] and ethoxymethylenemalononitrile. The presence of CN and $NH_2$ groups in the spectral data of compound **2** assigned its structure (cf. Experimental). When compound **2** was refluxed with triethyl orthoformate, it afforded N-[4-cyano-2-(6-phenyl-pyridazin-3-yl)-2H-pyrazol-3-yl]-formimidic acid ethyl ester (**3**). The IR

spectrum of compound **3** showed absorption band for cyano group, while the absorption band characteristic for the $NH_2$ group disappeared. Also, its $^1H$ NMR spectrum revealed the presence of $OCH_2CH_3$ and N═CH–O signals. The synthesis of 4-amino pyrimidine derivative **4** was achieved by reacting compound **3** with ammonium hydroxide solution (25%) to give 1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine (**4**) (Scheme 1). The IR spectrum of compound **4** did not show the absorption band of the cyano group and showed absorption band for $NH_2$. Its $^1H$ NMR spectrum revealed band for $NH_2$, $D_2O$ exchangeable. Benzoylation of compound **4** with benzoyl chloride, in dry dioxane, yielded *N*-[1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-benzamide (**5**). The IR spectrum of compound **5** showed absorption bands for NH and C═O. Its $^1H$ NMR spectrum revealed band at 11.90 (s, 1H, NH, $D_2O$ exchangeable).

The synthesis of methyl-[1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-amine (**6**) was achieved by treatment of compound **3** with alcoholic methylamine (35% in methanol). The IR and $^1H$ NMR spectra of compound **6** revealed signals for $NHCH_3$.

When compound **3**, in dry ethanol, was stirred with hydrazine hydrate at room temperature, it afforded 4-imino-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo[3,4-*d*] pyrimidin-5-ylamine (**7**) (Scheme 1). The latter compound was isomerized to the corresponding more thermodynamically stable [1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl]-hydrazine (**8**), upon refluxing in dry dioxane in the presence of few drops of piperidine. Actually,

**Scheme 1**



piperidine acts as a base in this Dimroth rearrangement reaction, and the IR spectrum of compounds **7** and **8** did not show any absorption band for the cyano group, whereas the IR and $^1$H NMR spectra showed absorption bands for NH and $NH_2$ groups.

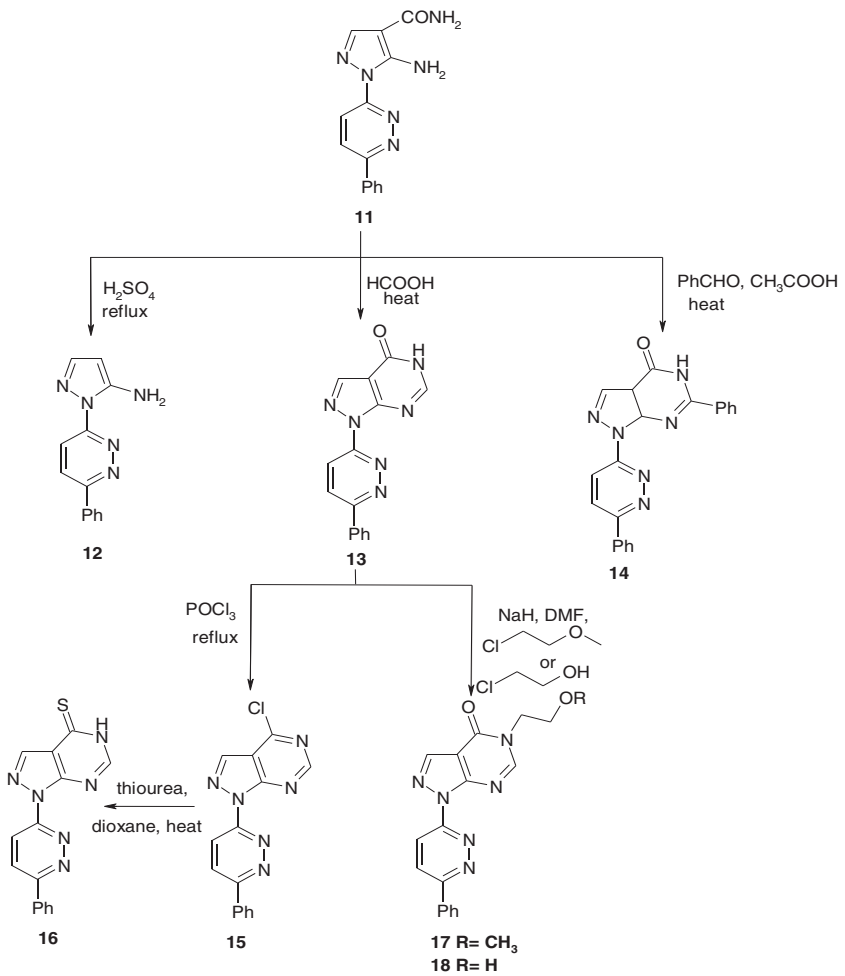
Acetylation of compound **2** with acetic anhydride in dry pyridine yielded *N*-[4-cyano-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazol-3-yl]-acetamide (**9**) (Scheme 1). In an attempt to cyclize compound **9** to pyrimidine derivative **10** by stirring in a mixture of polyphosphoric acid (PPA) and orthophosphoric acid (1:1) at room temperature, the unexpected 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid amide (**11**) was obtained via deacetylation of *N*-acetyl group besides the partial hydrolysis of cyano group. The $^1$H NMR spectrum showed the absorption bands of the two $NH_2$ as well as C=O in IR spectrum. Also, compound **11** was obtained directly by stirring of compound **2** with concentrated sulfuric acid (98%).

Although further heating of compound **11** in sulfuric acid (98%) for 2 h afforded 2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazol-3-ylamine (**12**) (Scheme 2). The IR (KBr) spectrum of compound **12** did not show the absorption bands of the cyano group or carbonyl group and showed absorption band for ($NH_2$). The sequence of the formation of compound **12** presumably took place via complete hydrolysis of cyano group followed by decarboxylation.

The pyrazolo[3,4-*d*]pyrimidin-4-ones **13** and **14** could be obtained directly by refluxing compound **11** with formic acid or benzaldehyde in glacial acetic acid, respectively. The IR and $^{13}$C NMR spectra of compounds **13** and **14** showed absorption bands for C=O and their $^1$H NMR spectra showed signals at 12.50 and 12.70 (s, 1H, NH, $D_2O$ exchangeable).

Compound **13** was converted to its corresponding 4-chloro derivative by refluxing with phosphorus oxychloride to give 4-chloro-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo [3,4-*d*]pyrimidine (**15**) (Scheme 2). The mass spectrum of compound **15** showed the molecular ion peak $M^+$ at m/z 308 as the base peak as well as the presence of isotopic pattern of chlorine atom. The pyrazolo[3,4-*d*]pyrimidine-

**Scheme 2**



4-thione **16** was obtained by refluxing compound **15** with thiourea in dry dioxane. The spectral data of compound **16** was in agreement with the proposed structure, in particular, the $^{13}$C NMR spectrum that revealed C═S at 170 ppm (cf. Experimental).

The synthesis of pyrimidine and fused heterocyclic pyrimidine nucleosides has been extensively reported in the literature [19,20]. However, there is still a great need and interest to prepare more active acyclic pyrimidine nucleosides with fewer side effects than those observed for the already known derivatives. Therefore, when the sodium salt of compound **13** (generated *in situ*) was treated with 2-chloroethyl methyl ether or 2-chloroethanol, it afforded the corresponding *N*-acyclic nucleosides **17** and **18**, respectively. The structure of the aforementioned acyclic nucleosides was confirmed with spectral data and their $^{1}$H NMR spectra revealed methoxyethyl and hydroxyethyl signals. In addition, the IR and $^{13}$C NMR spectra revealed that the site of attack was on the N-atom and not O-atom (cf. Experimental).

## BIOLOGICAL EVALUATION

Antimicrobial activity of newly synthesized compounds **2–9** and **11–18** was tested in concentration of 0.1 g/mL by using dimethylformamide as a solvent.

## CONCLUSIONS

Among the tested compounds, it was noticed that pyrazole derivatives (compounds **3** and **9)** and acyclic nucleosides (compounds **17** and **18**) showed more significant antimicrobial activity than the other tested compounds and some known drugs (standers).

| Tested compounds and standers | Inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Micro-organism | | | |
| | Bacteria | | | |
| | Gram-positive | Gram-negative | Yeast | Fungi |
| | *Escherichia coli* | *Bacillus subtilis* | *Aspergillus niger* | *Candida albicans* |
| Streptomycin | +++ | +++ | + | +++ |
| Erythromycin | – | +++ | – | – |
| Ampicillin | ++ | – | – | – |
| Amoxicillin | ++ | – | – | – |
| Fusidic acid | – | – | +++ | +++ |
| **2** | – | ++ | ++ | ++ |
| **3** | +++ | ++ | +++ | +++ |
| **4** | + | ++ | + | ++ |
| **5** | + | + | ++ | + |
| **6** | – | – | ++ | ++ |
| **7** | + | ++ | + | ++ |
| **8** | – | – | ++ | ++ |
| **9** | +++ | +++ | +++ | ++ |
| **11** | – | ++ | ++ | – |
| **12** | ++ | – | – | ++ |
| **13** | – | – | ++ | ++ |
| **14** | – | – | ++ | ++ |
| **15** | – | ++ | ++ | – |
| **16** | – | + | + | + |
| **17** | +++ | ++ | ++ | +++ |
| **18** | +++ | ++ | ++ | +++ |

+++Highly sensitive (inhibition zone = 21–25 mm).
++Fairly sensitive (inhibition zone = 16–20 mm).
+Slightly sensitive (inhibition zone = 10–15 mm).
–Not sensitive.

## EXPERIMENTAL

All melting points are uncorrected and measured using Electro-Thermal IA 9100 apparatus (Shimadzu, Japan). Infrared spectra were recorded as potassium bromide pellets on a PerkinElmer 1650 spectrophotometer (PerkinElmer Inc., Norwalk, CT), National Research Center, Cairo, Egypt. $^{1}$H NMR and $^{13}$C NMR spectra were determined on a Jeol-EX-500 NMR spectrometer (JEOL,

Tokyo, Japan) and chemical shifts were expressed as parts per million; ($\delta$ values, ppm) against TMS as internal reference, National Research Center, Cairo, Egypt. Mass spectra were recorded on EI + Q1 MSLMR UPLR, National Research Center, Cairo, Egypt. Microanalyses were operated using Electrothermal IA 9100 apparatus, (Electrothermal, Essex, U.K.), Organic Microanalysis Unit, National Research Center, Cairo, Egypt. Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Compound **1** was prepared according to a reported method [18].

**5-Amino-1-(6-phenyl-pyridazin-3-yl)-1H-pyrazole-4-carbonitrile (2).** To a solution of 1.86 g (0.01 mole) of compound **1** in 30 mL ethanol, 0.01 mole of ethoxymethylenemalononitrile was added. The reaction mixture was refluxed for 2 h. The formed precipitate was filtered on hot, dried, and recrystallized from dioxane to give compound **2** (2.52 g, 96%); m.p. 251–252°C. IR spectrum (KBr, ν, $cm^{-1}$): 3394, 3291 ($NH_2$) and 2221 (CN); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 7.50–7.70 (m, 3H, Ar–H), 7.80 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.00 (s, 1H, pyrazole-H), 8.10−8.30 (m, 3H, 2 Ar–H + pyridazine-H) and 8.45 (d, $J$ = 9.0 Hz, 1H, pyridazine-H); MS, *m/z* (%): 262 ($M^+$, 100), 233 (2.47), 197 (22.30), 185 (1.20), 155 (1.85), 140 (10.60), 115 (1.85), 104 (3.53), 102 (5.33), 93 (1.16), 89 (2.43), 77 (7.01). *Anal*. Calcd for $C_{14}H_{10}N_6$ (262.27): C, 64.11; H, 3.84; N, 32.05; Found: C, 64.33; H, 3.70; N, 31.93.

**N-[4-Cyano-2-(6-phenyl-pyridazin-3-yl)-2H-pyrazol-3-yl]-formimidic acid ethyl ester (3).** Compound **2**, 2.62 g (0.01 mol) was refluxed in 40 mL triethyl orthoformate for 5 h. The product that separated on cooling was filtered off and recrystallized from dry dioxane to give compound **3** (2.98 g, 94%); m.p. 170–172°C. IR spectrum (KBr, ν, $cm^{-1}$): 2229 (CN); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 1.30 (t, $J$ = 7.3 Hz, 3H, $OCH_2CH_3$), 4.30 (q, $J$ = 7.3 Hz, 2H, $OCH_2CH_3$), 7.50–7.70 (m, 3H, Ar–H), 8.10–8.40 (m, 4H, 2 Ar–H + pyrazole-H + pyridazine-H), 8.50 (d, $J$ = 9.0 Hz, 1H, pyridazine-H), and 8.65 (s, 1H, N = CH–O); MS, *m/z* (%): 318 ($M^+$, 14.11), 289 (100), 273 (19.81), 197 (4.61), 155 (8.19), 140 (6.48), 115 (8.03), 102 (4.00), 77 (11.95). *Anal*. Calcd for $C_{17}H_{14}N_6O$ (318.34): C, 64.14; H, 4.43;N, 26.39; Found: C, 64.21; H, 4.28; N, 26.40.

**1-(6-Phenyl-pyridazin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamine (4).** Ammonium hydroxide solution (20 mL, 25%) was added to 20 mL absolute ethanol containing 3.18 g (0.01 mol) of compound **3**, and the reaction mixture was stirred at room temperature for 2 h. The formed precipitate was filtered off, dried, and recrystallized from dioxane to give compound **4** (2.57 g, 89%), m.p. 300–301°C. IR spectrum (KBr, ν, $cm^{-1}$): 3384, 3079 ($NH_2$); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 7.50–7.70 (m, 3 H, Ar–H), 7.80–8.30 (m, 4H, $NH_2$, $D_2O$ exchangeable + 2Ar–H), 8.40 (s, 1H, pyrazole-H) and 8.50–8.60 (m, 3H, 2 pyridazine-H + pyrimidine-H); MS, *m/z* (%): 289 ($M^+$, 100), 262 (33.29), 235 (13.84), 206 (6.77), 179 (2.24), 140 (8.60), 115 (23.93), 77 (5.09). *Anal*. Calcd for $C_{15}H_{11}N_7$ (289.28): C, 62.27; H, 3.83; N, 33.89; Found: C, 62.39; H, 3.74; N, 33.87.

**N-[1-(6-Phenyl-pyridazin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]-benzamide (5).** To a solution of 2.89 g (0.01 mol) of compound **4** in 50 mL dry dioxane, 1.4 mL (0.01 mol) benzoyl chloride was added. The mixture was allowed to heat for 3 h, and the excess solvent was removed under reduced pressure leaving an oil that was washed with benzene to give a brown solid. The remaining product was purified on silica gel using ethyl acetate: pet. ether (40–60°C) (2:1) as eluent to give compound **5** (2.44 g, 62%), m.p. 233–235°C. IR spectrum (KBr, *v*, $cm^{-1}$): 3244 (NH) and 1698 (CO); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 7.50–7.70 (m, 6H, Ar–H), 8.10–8.30 (m, 4H, Ar–H), 8.50–8.60 (m, 2H, 2 pyridazine-H), 8.80 (s, 1H, pyrazole-H), 9.00 (s, 1H, pyrimidine-H) and 11.90 (s, 1H, NH, $D_2O$ exchangeable); MS, *m/z* (%): 393 ($M^+$, 37.06), 364 (100), 316 (3.26), 289 (14.95), 262 (6.81), 234 (3.08), 140 (4.89), 115 (12.62), 105 (71.97), 77 (45.62). *Anal*. Calcd for $C_{22}H_{15}N_7O$ (393.39): C, 67.16; H, 3.84; N, 24.92; Found: C, 67.13; H, 3.66; N, 25.05.

**Methyl-[1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl]-amine (6).** Methylamine solution (20 mL, 35%) was added to 20 mL absolute ethanol containing 3.18 g (0.01 mol) of compound **3**, then the reaction mixture was stirred at room temperature for 8 h. The formed precipitate was filtered off, washed with ethanol, dried, and recrystallized from dimethyl formamide to give compound **6** (2.34 g, 77%); m.p. 259–261°C. IR spectrum (KBr, ν, $cm^{-1}$): 3283 (NH); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 3.00 (s, 3H, $CH_3$), 7.50–7.65 (m, 3H, Ar–H), 8.10–8.25 (m, 2H, Ar–H) and 8.30–8.70 (m, 5H, NH, $D_2O$ exchangeable + 2 pyridazine-H + pyrazole-H + pyrimidine-H); MS, *m/z* (%): 303 ($M^+$, 100), 288 (7.77), 276 (18.42), 247 (31.97), 220 (8.89), 206 (4.43), 144 (5.80), 115 (34.01), 77 (15.61). *Anal*. Calcd for $C_{16}H_{13}N_7$ (303.32): C, 63.35; H, 4.31; N, 32.32, Found: C, 63.02; H, 4.49; N, 32.43.

**4-Imino-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo [3,4-*d*]pyrimidin-5-ylamine (7).** To a solution of 3.18 g (0.01 mol) of compound **3** in 30 mL dry ethanol, 3 mL hydrazine hydrate (99%) was added with stirring for 1 h at room temperature. The obtained product was filtered, dried, and recrystallized from dry dioxane to give compound **7** (2.80 g, 92%); m.p. 310–311°C. IR spectrum (KBr, ν, $cm^{-1}$): 3410, 3358 ($NH_2$), and 3296 (NH); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm), 7.30–7.70 (m, 5H, 3Ar–H + $NH_2$, $D_2O$ exchangeable), 8.10 (s, 1H, pyrazole-H), 8.15–8.30 (m, 3H, 2Ar–H + pyridazine-H), 8.50 (d, $J$ = 9.0 Hz, 1H, pyridazine-H), 8.60 (s, 1H, pyrimidine-H), and 12.60 (s, 1H, NH, $D_2O$ exchangeable); MS, *m/z* (%): 304 ($M^+$, 100), 290 (22.17), 261 (4.25), 235 (3.60), 197 (23.65), 171 (11.52), 152 (4.33), 115 (15.50), 102 (8.42), 77 (9.04). *Anal*. Calcd for $C_{15}H_{12}N_8$ (304.32): C, 59.20; H, 3.97; N, 36.82; Found: C, 59.16; H, 3.79; N, 36.99.

**[1-(6-Phenyl-pyridazin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-hydrazine (8).** Compound **7** (1.52 g, 0.005 mol) in 20 mL dry dioxane containing few drops of piperidine was refluxed for 6 h. Then the reaction mixture was evaporated under reduced pressure and recrystallized from dioxane to give compound **8** (1.03 g, 68%), m.p. 337–338°C. IR spectrum (KBr, ν, $cm^{-1}$): 3425, 3387 ($NH_2$), and 3310 (NH); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 6.40 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.50–7.65 (m, 3H, Ar–H), 7.80 (s, 1H, NH, $D_2O$ exchangeable) and 8.10–8.60 (m, 6H, 2 Ar–H + pyrazole-H + 2 pyridazine-H + pyrimidine-H); MS, *m/z* (%): 304 ($M^+$, 5.19), 262 (100), 248 (1.57), 237 (1.49), 233 (3.23), 197 (38.35), 171 (9.96), 140 (15.58), 115 (20.71), 102 (15.75), 77 (13.76). *Anal*. Calcd for $C_{15}H_{12}N_8$ (304.32): C, 59.20; H, 3.97; N, 36.82; Found: C, 59.25; H, 3.83; N, 36.88.

***N*-[4-Cyano-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazol-3-yl]-acetamide (9).** To a solution of compound **2** (2.62 g, 0.01 mol) in 20 mL dry pyridine, 10 mL of acetic anhydride was added. The reaction mixture was maintained at room temperature for 24 h, which afforded no change, and then heated at 50°C for 6 h, then was evaporated under reduced pressure, and the remaining oil product was purified with column chromatography (silica gel) using pet. ether (40–60°C):ethyl acetate mixture (1:1), as eluent to give compound **9** (2.10 g, 69%); m.p. 283–285°C. IR spectrum (KBr, ν, $cm^{-1}$): 3256 (NH), 2234 (CN) and 1702 (CO); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 2.10 (s, 3H, $CH_3$), 7.55–7.65 (m, 3H, Ar–H), 8.20–8.30 (m, 3H, 2Ar–H + pyridazine-H), 8.50

(s, 1H, pyrazole-H), 8.60 (d, $J = 9.0$ Hz, 1H, pyridazine-H), and 11.20 (s, 1H, NH, $D_2O$ exchangeable); MS, *m/z* (%): 304 ($M^+$, 11.13), 278 (3.13), 262 (100), 233 (1.54), 197 (16.12), 140 (5.24), 115 (5.94), 102 (2.84), 77 (5.54). *Anal*. Calcd for $C_{16}H_{12}N_6O$ (304.31): C, 63.14; H, 3.97; N, 27.61; Found: C, 62.93; H, 4.02; N, 27.70.

**5-Amino-1-(6-phenyl-pyridazin-3-yl)-1H-pyrazole-4-carboxylic acid amide (11).**

**Method A**: A solution of 1.52 g (0.005 mol) of compound **9** in a mixture of PPA and orthophosphoric acid (1:1) was stirred at room temperature for 6 h. The reaction mixture was poured into water, and the organic material was extracted with ethyl acetate. The solvent was removed under reduced pressure leaving a solid product that was recrystallized from dioxane to give compound **11** (0.82 g, 59%), m.p. 273–275°C.

**Method B**: To 10 mL cold concentrated sulfuric acid (98%), 1.31 g (0.005 mol) of compound **2** was added, over a 5 min period with ice bath cooling and stirring. 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 pour onto ice chips and the resulting precipitate was collected, washed with water, dried, and recrystallized from dioxane to give a compound (1.13 g, 80.71%) identical in all aspects with compound **11** (m.p., mixed m.p., TLC, and IR); m.p. 273–274°C. IR spectrum (KBr, ν, $cm^{-1}$): 3440, 3324, 3200, 3120 ($2NH_2$), and 1654 (CO); $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 6.95 (bs, 2H, $NH_2$, $D_2O$ exchangeable) 7.40–7.90 (m, 5H, 3Ar–H + $NH_2$, $D_2O$ exchangeable), 8.10 (s, 1H, pyrazole-H), 8.15–8.35 (m, 3H, 2Ar–H + pyridazine-H), and 8.50 (d, $J = 9.0$ Hz, 1H, pyridazine-H); MS, *m/z* (%): 280 ($M^+$, 100), 263 (43.0), 236 (3.0), 197 (26.0), 156 (4.0), 140 (8.0), 115 (3.50). *Anal*. Calcd for $C_{14}H_{12}N_6O$ (280.28): C, 59.99; H, 4.31; N, 29.98; Found: C, 59.87; H, 4.38; N, 29.95.

**2-(6-Phenyl-pyridazin-3-yl)-2H-pyrazol-3-ylamine (12).** Compound **2**, 1.31 g (0.005 mol), in 20 mL sulfuric acid (50%) was refluxed for 2 h. The reaction mixture was cooled and poured onto ice chips and the resulting precipitate was collected, washed with water, dried, and purified with column chromatography (silica gel) using chloroform: n-hexane (3:1) as eluent to give compound **12** (0.58 g, 49%), m.p. 151–152°C. IR spectrum (KBr, ν, $cm^{-1}$): 3408, 3303 ($NH_2$); $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 5.50 (s, 1H, pyrazole-H), 7.00 (s, 2H, $NH_2$, exchangeable with $D_2O$), 7.40–7.60 (m, 4H, 3Ar–H + pyrazole-H), 8.10–8.30 (m, 3H, 2Ar–H + pyridazine-H), and 8.40 (d, $J = 8.55$ Hz, 1H, pyridazine-H); MS, *m/z* (%): 237 ($M^+$, 100), 221 (2.61), 197 (32.35), 181 (2.71), 171 (2.02), 155 (4.05), 140 (24.30), 115 (22.22), 102 (5.30), 77 (12.99). *Anal*. Calcd for $C_{13}H_{11}N_5$ (237.26): C, 65.80; H, 4.67; N, 29.52; Found: C, 65.89; H, 4.69; N, 29.33.

**1-(6-Phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-d] pyrimidin-4-one (13)**. Compound **11**, 1.4 g (0.005 mol) in 20 mL formic acid (85%) was refluxed for 5 h. The reaction mixture was cooled and poured into water, and the formed solid was filtered off, dried, and recrystallized from dimethylformamide to give a compound **13** (1.40 g, 79%); m.p. 345–346°C. IR spectrum (KBr, ν, $cm^{-1}$): 3058 (NH), and 1665 (CO); $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 7.55–7.70 (m, 3H, Ar–H), 8.20–8.30 (m, 3H, 2Ar–H + pyrazole-H), 8.37 (d, $J = 9.0$ 1H, pyridazine-H), 8.50–8.60 (m, 2H, pyridazine-H + pyrimidine-H), and 12.50 (s, 1H, NH, $D_2O$ exchangeable); $^{13}$C NMR spectrum (DMSO-$d_6$, δ ppm): 108 (C3a), 137 (C3), 140 (C6), 159 (C7a), 159 (C═O), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 118, 121, 130, and 132 (C-phenyl). MS, *m/z* (%): 290 ($M^+$, 100), 261 (18.49), 235 (6.80), 206 (15.14), 179 (5.23), 140 (11.60), 115 (18.88), 102 (16.83), 77 (6.61). *Anal*. Calcd for $C_{15}H_{10}N_6O$ (290.27): C, 62.06; H, 3.47; N, 28.95; Found: C, 61.77; H, 3.56; N, 29.08.

**1-(6-Phenyl-pyridazin-3-yl)-6-phenyl-1,5-dihydro-pyrazolo [3,4-d]-pyrimidin-4-one (14)**. An equimolar amount of compound **11** (0.28 g, 0.001 mol) and benzaldehyde in 25 mL glacial acetic acid was refluxed for 10 h. The formed solid was filtered on hot, dried, and recrystallized from dimethylformamide to give compound **14** (0.24 g, 86%); m.p. 387–388°C. IR spectrum (KBr, ν, $cm^{-1}$): 3448 (NH) and 1687 (CO); $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 7.50–7.70 (m, 6H, Ar–H), 8.10–8.30 (m, 4H, Ar–H), 8.50–8.70 (m, 3H, pyrazole-H + 2 pyridazine-H) and 12.70 (s, 1H, NH, $D_2O$ exchangeable); $^{13}$C NMR spectrum (DMSO-$d_6$, δ ppm): 108 (C3a), 137 (C3), 140 (C6), 159 (C7a), 161 (C═O), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 118, 119,120, 121, 124, 130, and 132 (C-phenyl). MS, *m/z* (%): 366 ($M^+$, 100), 262 (85.45), 206 (5.74), 179 (1.37), 140 (9.80), 115 (14.24), 104 (69.35), 77 (5.46). *Anal*. Calcd for $C_{21}H_{14}N_6O$ (366.37): C, 68.84; H, 3.85; N, 22.94; Found: C, 68.62; H, 3.98; N, 22.88.

**4-Chloro-1-(6-phenyl-pyridazine-3-yl)-1*H*-pyrazolo[3,4-*d*] pyrimidine (15)**. Compound **13** (1.45 g, 0.005 mol) in 10 mL phosphorus oxychloride was refluxed for 10 h, then the reaction mixture was cooled and poured into ice-water. The formed solid was filtered off, dried, and purified on silica gel using chloroform:n-hexane (1:1) as eluent to give compound **15** (0.85 g, 55%) yield, m.p. 264–266°C. $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 7.55–7.70 (m, 3H, Ar–H), 8.20–8.30 (m, 2H, Ar–H), 8.50 (d, $J = 9.0$ Hz, 1H, pyridazine-H), 8.62 (d, $J = 9.0$ Hz, 1H, pyridazine-H), 9.00 (s, 1H, pyrazole-H), and 9.10 (s, 1H, pyrimidine-H); MS, *m/z* (%): 310 ($M^+$, $Cl^{37}$, 38.41), 308 ($M^+$, $Cl^{35}$, 100), 244 (11.11), 218 (11.28), 190 (4.88), 140 (14.02), 115 (25.35). 101 (20.93), 77 (8.15). *Anal*. Calcd for $C_{15}H_9ClN_6$ (308.73): C, 58.35; H, 2.93; N, 27.22; Found: C, 58.30; H, 2.94; N, 27.26.

**1-(6-Phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*] pyrimidine-4-thione (16)**. Compound **15** (1.54 g, 0.005 mol) and thiourea (0.38 g, 0.005 mol) in 40 mL dry dioxane was refluxed for 4 h. The formed precipitate was collected and dissolved in NaOH (20 mL, 10%). Then the mixture was filtered off, and the filtrate was precipitated with HCl (0.1 *N*). The solid product was collected and recrystallized from dimethylformamide to give a compound **16** (1.16 g, 76%) m. p. 336–337°C. IR spectrum (KBr, ν, $cm^{-1}$): 3025 (NH), and 1365 (C═S); $^1$H NMR spectrum (DMSO-$d_6$, δ ppm): 7.55–7.65 (m, 3H, Ar–H), 8.15–8.30 (m, 2H, Ar–H), 8.35–8.45 (m, 2H, pyrazole-H + pyridazine-H), 8.50–8.60 (m, 2H, pyridazine-H + pyrimidine-H), and 14.00 (s, 1H, NH, $D_2O$ exchangeable); $^{13}$C NMR spectrum (DMSO-$d_6$, δ ppm): 107 (C3a), 137 (C3), 141 (C6), 159 (C7a), 170 (C═S), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 118, 121, 130, and 132 (C-phenyl). MS, *m/z* (%): 306 ($M^+$, 100), 277 (6.05), 272 (1.13), 252 (5.29), 218 (1.33), 176 (1.79), 140 (2.18), 115 (8.64), 102 (2.72), 77 (1.99). *Anal*. Calcd for $C_{15}H_{10}N_6S$ (306.34): C, 58.80; H, 3.29; N, 27.43; S, 10.46; Found: C, 58.92; H, 3.33; N, 27.10; S, 10.57.

## GENERAL PROCEDURE FOR SYNTHESIS OF COMPOUNDS **17** AND **18**

To a solution of compound **13** (0.01 mol) in dry DMF (50 mL), 50% oil-immersed sodium hydride (0.20 g) was added. Thereafter, the reaction mixture was stirred at room temperature for 1 h. Then, 2-chloroethyl methyl ether or 2-chloroethanol (0.02 mol) was added and the reaction mixtures were stirred at 70°C for 3 and 5 h, respectively. After evaporation under reduced pressure, the residues were purified on silica gel column using chloroform: methanol (9:1) as an eluent to give compounds **17** and **18**, respectively.

**5-(2-Methoxyethyl)-1-(6-phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (17)**. (1.49 g, 43%); m.p. 219–220°C. IR spectrum (KBr, ν, $cm^{-1}$): 1691 (C═O); $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 3.30 (s, 3H, $OCH_3$), 3.65 (t, *J* = xx Hz, 2H, $CH_2O$), 4.10–4.25 (t, *J* = xx Hz, 2H, $NCH_2$), 7.50–7.75 (m, 3H, Ar–H), 8.15–8.30 (m, 2H, Ar–H), 8.37 (d, *J* = 9.0 Hz, 1H, pyridazine-H), and 8.45–8.60 (m, 3H, pyridazine-H + pyrazole-H + pyrimidine-H); $^{13}C$ NMR spectrum ($DMSOd_6$,δ ppm): 32 ($CH_2O$), 58 ($OCH_3$), 69 ($CH_2N$), 108 (C3a), 137 (C3), 140 (C6), 159 (C7a), 162 (C═O), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 118, 121, 130, and 132 (C-phenyl); MS, *m/z* (%): 378 (M+, 8.35), 333 (17), 320 (100). MS, *m/z* (%): 348 ($M^+$, 18.93), 303 (4.66), 290 (100), 261 (16.28), 248 (3.29), 235 (6.11), 155 (4.09), 140 (2.77), 115 (6.45), 77 (4.25). *Anal*. Calcd for $C_{18}H_{16}N_6O_2$ (348.36): C, 62.06; H, 4.63; N, 24.13; Found: C, 62.09; H, 4.41; N, 24.23.

**5-(2-Hydroxyethyl)-1-(6-phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (18)**. (1.60 g, 48%); m.p. 293–295°C. The IR spectrum (KBr, ν, $cm^{-1}$): 3415–3350 (OH) and 1722 (C═O). Its $^1H$ NMR spectrum (DMSO-$d_6$, δ ppm): 3.14–3.21 (m, 2H, $CH_2O$), 3.73 (t, *J* = xx Hz, 2H, $CH_2N$), 5.05 (s, 1H, OH, $D_2O$ exchangeable), 7.65–7.69 (m, 3H, Ar–H), 8.28–8.39 (m, 2H, Ar–H), 8.42–8.59 (m, 4H, 2 pyridazine-H + pyrazole-H + pyrimidine-H). $^{13}C$ NMR spectrum ($DMSOd_6$,δ ppm): 31 ($CH_2O$), 63 ($CH_2N$), 108 (C3a), 137 (C3), 140 (C6), 159 (C7a), 158 (C═O), 122, 126, 153, and 156 (C5, C4, C6, and C3 pyridazine), 113, 118, 121, 130, and 132 (C-phenyl); MS, *m/z*(%): 334 ($M^+$, 47.63), 303 (6.07), 290 (100), 261 (26.11), 248 (5.11), 235 (10.94), 207 (10.08), 198 (3.22), 179 (5.28), 155 (9.75), 140 (13.01), 115 (29.67), 101 (18.35), 77 (19.92). *Anal*. Calcd for $C_{17}H_{14}N_6O_2$ (334.33): C, 61.06; H, 4.22; N, 25.13; Found: C, 61.17; H, 4.06; N, 25.18.

## BIOLOGICAL EVALUATION

Micro-organisms' species:

1) Bacteria
   a) Gram-negative bacteria, *Bacillus subtilis*
   b) Gram-positive bacteria, *Escherichia coli*
2) Yeast: *Candida albicans*
3) 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 milliliter of the spore suspension of each micro-organism was spread all over the surface of the cold solid medium placed in the petri-dish.

**Method [21].** A total of 0.5 g of each tested compounds was dissolved in 5 mL of dimethylformamide. An amount of 0.1 mL of test solution was placed on Whatman 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 three discs. The petri-dishes were incubated at 5°C for an hour to permit good diffusion and then transferred to an incubator of 85°C overnight, then examined. The results were then recorded by measuring the inhibition zone diameters.

**Acknowledgments.** The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-032.

## REFERENCES AND NOTES

[1] Larsen, J. S.; Zahran, M. A.; Pedersen, E. B.; Nielsen, C. Monatsh Chem 1999, 130, 1167.
[2] Rashad, A. E.; Shamroukh, A. H.; Abdel-Megeid, R. E.; Sayed, H. H.; Abdel-Wahed, N. A. Scientia Pharm 2010, 78, 1.
[3] Rashad, A. E.; Shamroukh, A. H.; Abdel-Megeid, R. E.; Mostafa, A.; Ali, M. A.; Banert, K. Nucleosides Nucleotides 2010, 29, 809.
[4] Bendich, P. J.; Russell, J. J.; Fox, J. Am Chem Soc 1954, 76, 6073.
[5] Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Akberali, P. M.; Shetty, N. S. Bioorg Med Chem 2006, 14, 2040.
[6] Shamroukh, A. H.; Rashad, A. E.; Sayed, H. H. Phosphorous, Sulfur and Silicon Relat Elem 2005, 180, 2347.
[7] Liu, H.; Wang, H. Q.; Liu, Z. J. Bioorg Med Chem Lett 2007, 17, 2203.
[8] Rashad, A. E.; Mahmoud, A. E.; Ali, M. A. Eur J Med Chem 2011, 46, 1019.
[9] Filler, R. Chem Technol 1974, 4, 752.
[10] EI-Feky, S. A.; Abd EI- Samii, Z. K. Pharmazie 1996, 51, 540.
[11] El-Hamouly, W. S.; El-Khamry, A. A.; Abbas, E. M. H. Indian J Chem 2006, 45B, 2091.
[12] Garg, N.; Avasthi, K.; Pratap, R.; Bhakuni, D. S.; Guru, P. Y. Indian J Chem 1990, 29B, 859.
[13] Vijaya Raj, K. K.; Narayana, B.; Ashalatha, B. V.; Suchetha Kumari, N. J Pharmacol Toxicol 2006, 1(6), 559.
[14] Amin, M. A.; Ismail EI-Helby, M. M.; Addel, G. A.; Bauomy, A. H.; EI-Morsy, M. A.; Alex J Pharm Sci 2003, 17, 1.
[15] Rashad, A. E.; Shamroukh, A. H.; Ali, M. A.; Abdel-Motti, F. M. Heteroatom Chem 2007, 18, 274.

[16] Abdel-Motti, F. M.; Shamroukh, A. H.; Sayed, H. H.; Abdel-Megeid, F. M. E. Egypt J Chem 2002, 45, 1123.

[17] Rashad, A. E.; Hegab, M. I.; Abdel-Megeid, R. E.; Fatahala, N. A.; Abdel-Megeid, F. M. E. Eur J Med Chem 2009, 44, 3285.

[18] Moustafa, A. H.; Kaddah, A. M.; Shams, N. A. Indian J Chem 1981, 20B, 1084.

[19] Hafez, H. N.; El-Gazzar, A. B. A.; Nawwar, G. A. M. Eur J Med Chem 2010, 45, 1485.

[20] Rashad, A. E.; Shamroukh, A. H.; Sayed, H. H.; Awad, S. M.; Abdel-Wahed, N. A. Synth Comm 2011, 41, 652.

[21] Abou-Zeid, A. A.; Shehata, Y. M. Indian J Pharm 1969, 31, 72.