Studies on the Reactivity of Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic Acid Hydrazide Towards Some Reagents for Biological Evaluation

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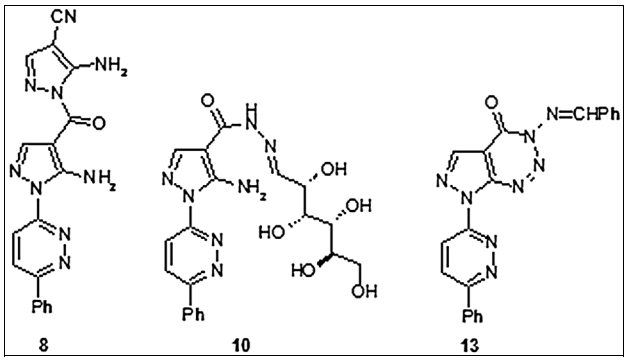

Novel 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid ethyl ester (**2**) was formed using (6-phenyl-pyridazin-3-yl)-hydrazine (**1**) and ethyl(ethoxymethylene)cyanoacetate. The β-enaminoester derivative **2** was in turn used as precursor for the preparation of 1-(6-phenyl-pyridazin-3-yl)-pyrazoles (**3**, **4**, **7–12**, **15**, **16**), 1-(6-phenyl-pyridazin-3-yl)-pyrazolo[3,4-*d*]pyrimidines (**5**, **6**, **14**) and 1-(6-phenyl-pyridazin-3-yl)-pyrazolo[3,4-*d*][1,2,3]triazine (**13**). The *in vitro* antimicrobial activity of the synthesized compounds was evaluated by measuring the inhibition zone diameters where some of them showed potent antimicrobial activity in compared with well-known drugs (standards).

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INTRODUCTION

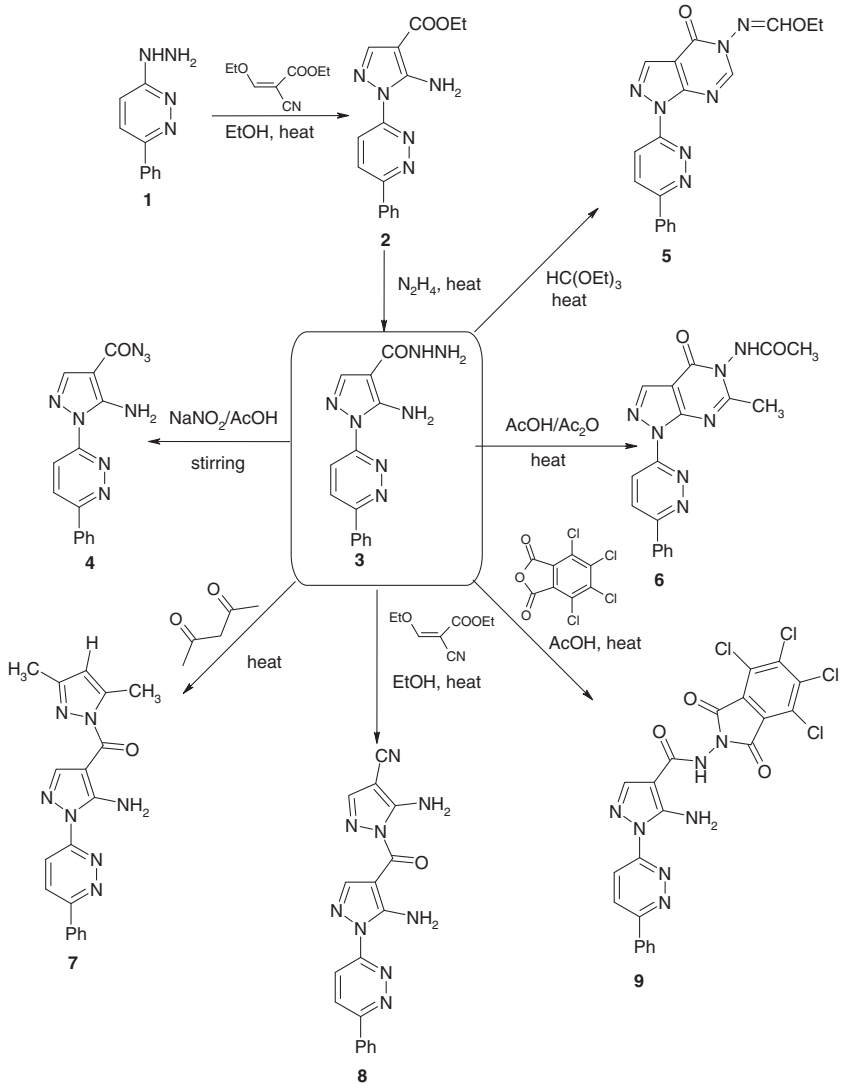
The chemistry of pyrazole derivatives has received a great attention because of their biological activities as HIV-1 inhibitors [1], HSV [2], bactericides [3], anticancer [4], and anti-inflammatory [5]. Also, considerable attention was devoted to the construction of new derivatives of pyrazolo[3,4-d]pyrimidines on the account of their reported biological activities [6–10]. Additionally, pyrazolo[3,4-d]pyrimidines are considered as biologically active isomeric purine analogs, because there is no much difference in the basic structure of pyrazolopyrimidines and purines where a pyrazole ring replaces the imidazole ring of purines [11,12]. In the light of the aforementioned facts, and as a continuation of our investigations on the synthesis of biologically active heterocyclic compounds [13–15], we were prompted to synthesize some new pyrazole and pyrazolo [3,4-d]pyrimidine derivatives to evaluate their potential antimicrobial activities.

RESULTS AND DISCUSSION

The starting material in this work, 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid ethyl ester (**2**), was prepared by refluxing a mixture of (6-phenyl-pyridazin-3-yl)-hydrazine (**1**) [16] and ethyl (ethoxymethylene)cyanoacetate in ethanol. IR spectrum of compound **2** showed absorption bands for (NH_2) and (CO) groups. Its ^{1}H NMR spectrum showed triplet and quartet signals of the CH_3 and CH_2 protons. Compound **2** was converted into corresponding acid hydrazide by heating with hydrazine hydrate (99%) to give 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid hydrazide (**3**). The IR spectrum of compound **3** showed absorption bands for (NH_2, NH), and (CO) groups. Its ^{1}H NMR spectrum revealed signals D_2O exchangeable at 4.30, 7.70, and 9.15 for 2 NH_2 and NH, respectively.

Compound **3** was converted into corresponding 4-carbonylazide derivative **4** by stirring of its solution, in acetic acid, with nitrous acid (prepared *in situ*) to give

Scheme 1



5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carbonylazide (**4**). The IR spectrum of compound **4** showed absorption bands at 2152 (N_3) and 1662 (C═O). When compound **3** was refluxed with triethyl orthoformate or a mixture of glacial acetic acid and acetic anhydride (1:1), it afforded *N*-[4-oxo-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo [3,4-*d*] pyrimidine-5-yl]formamidic acid ethyl ester (**5**) or *N*-[6-methyl-4-oxo-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo[3,4-*d*]pyrimidin-5-yl]acetamide (**6**), respectively. The IR spectrum of compound **5** did not show absorption bands for (NH, NH_2) and showed absorption band for (CO) group and its ^{1}H NMR spectrum showed signals of the CH_3 and CH_2 protons. The IR spectrum of compound **6** showed absorption bands for (NH) and two (CO) groups.

Also, when compound **3** was refluxed with acetylacetone or ethoxymethylenemalononitrile, it afforded [5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazol-4-yl]-(3,5-dimethylpyrazol-1-yl)-methanone (**7**) and [5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazol-4-yl]-(5-amino-4-cyano-pyrazol-1-yl)-methanone (**8**), respectively. The analytical and spectral data of the latter compounds are in agreement with the proposed structures (cf. Experimental).

When compound **3** was refluxed with tetrachlorophthalic anhydride (1:1) in glacial acetic acid, it afforded 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid-*N*-(4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydroisoindol-2-yl)-amide (**9**). The IR spectrum of compound **9** showed absorption bands for (NH_2, NH), anhydride C═O, and amide C═O groups. In addition, the ^{1}H NMR spectrum revealed signal at 12.60 (s, 1H, NH, D_2O exchangeable). Scheme 1.

Condensation of compound **3** with D-glucose in the presence of few drops of glacial acetic acid at 80 °C gave the corresponding aldehydo-sugar: 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid (2,3,4,5,6-

pentahydroxy-hexylidine)-hydrazide (**10**). Their IR and ^{1}H NMR spectra showed the presence of the sugar protons, NH, and azo-methine (CH═N) (cf. Experimental). When compound **10** was stirred with acetic acid/acetic anhydride mixture in pyridine at room temperature, it gave the *O*-acetylated sugar derivative **11**. The absence of OH in IR and ^{1}H NMR spectra confirmed its structure (cf. Experimental). Also, the ^{13}C NMR spectrum of compound **11** showed signals accountable for the acetylated sugar residue (cf. Experimental).

Also, condensation of compound **3** with benzaldehyde in the presence of few drops of piperidine gave 5-amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid benzylidene-hydrazide (**12**). The latter compound was annulated to a triazinone **13** and pyrazolo[3,4-*d*]pyrimidinone **14** by stirring with nitrous acid or refluxing with triethyl orthoformate, respectively. The IR spectra of compounds **13** and **14** did not show the absorption bands of (NH, NH_2) groups and showed absorption band for (CO) group.

Moreover, compound **12** was cyclized by refluxing in 1,4-dichlorobenzene to give 4-(5-phenyl-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazol-3-ylamine (**15**). Compound **12** underwent 1,5-dipolar cycloaddition by using bromine in glacial acetic acid in the presence of anhydrous sodium acetate (according to the method of Bansal *et al.*) [17] to give 4-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazole-3-ylamine (**16**). Also, the latter compound was obtained by dehydrogenation of compound **15** using bromine in acetic acid. Analytical and spectral data of these compounds are in agreement with the proposed structures (cf. Experimental). Scheme 2.

BIOLOGICAL EVALUATION

The *in vitro* antimicrobial activity of the newly synthesized compounds was tested, and the results are shown in Table 1. Evaluation of the new compounds established that

Scheme 2

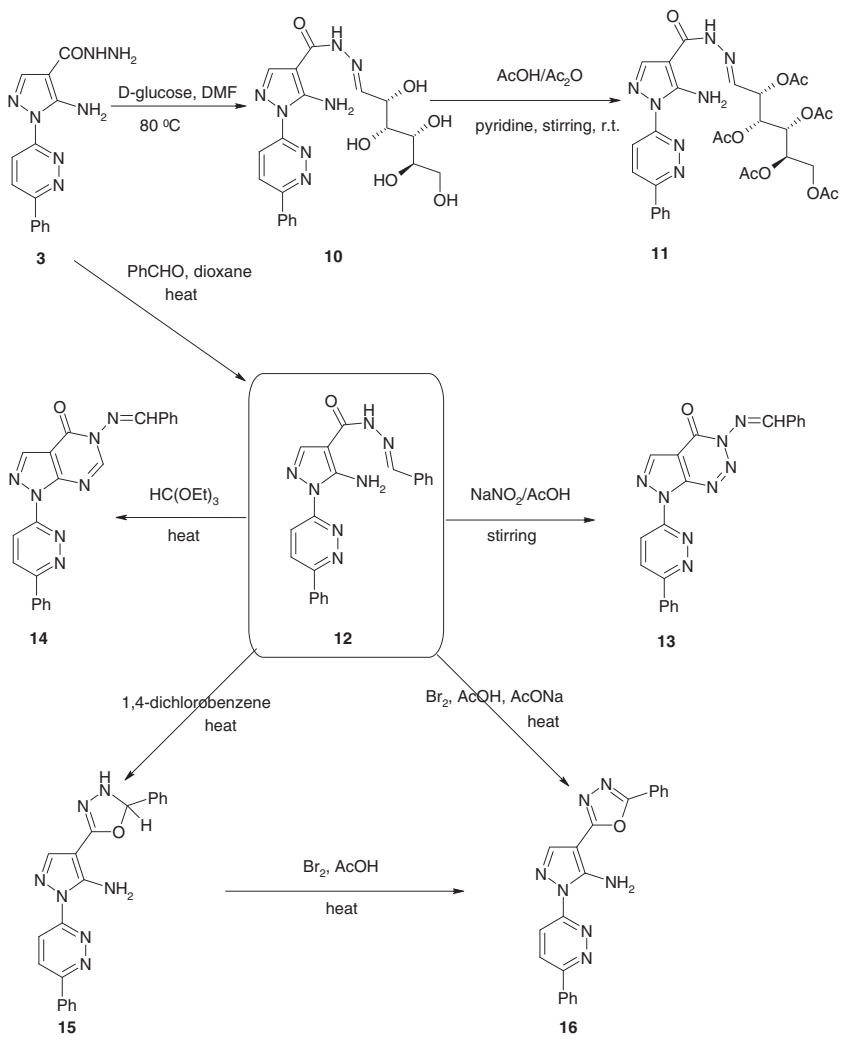


Table 1

Antimicrobial activity of some synthesized compounds.[a]

Tested Compd. No. and Standard	Disk diffusion test (mm)			
	Micro-organisms			
	Bacteria			
	Gram negative	Gram positive	Yeast	Fungi
	Escherichia coli	*Bacillus subtillis*	*Candida albicans*	*Aspergillus niger*
Streptomycin[b]	+++	+++	+++	+
2	+++	+++	+++	+
3	++	++	++	+
4	+	+	+	+
5	–	–	–	–
6	–	–	–	–
7	++	++	++	+
8	+++	+++	+++	+
9	+	+	+	–
10	+++	+++	+++	+
11	++	++	++	+
12	++	++	++	+
13	+++	+++	+++	+
14	–	–	–	–
15	–	–	–	–
16	+	+	+	+

[a] $\gamma = 2\ \mu g/mL^{-1}$ in DMSO.
[b] $\gamma = 25\ \mu g/mL^{-1}$ in DMSO, Lot. 30730, Bioanalyse (Turkey).
+++ highly sensitive (14–16 mm); ++ fairly sensitive (12–14 mm); + slightly sensitive (10–12 mm); – not sensitive.

compounds **2**, **8**, **10**, and **13** revealed higher activity against the micro-organisms (Gram positive, Gram negative bacteria and yeast) than the other tested compounds. On the other hand, it was found that compounds **3**, **7**, **11**, and **12** revealed fairly activity against the same micro-organisms.

CONCLUSIONS

Synthesis and structure characterization of pyrazoles (**3**, **4**, **7–12**, **15**, **16**), pyrazolo[3,4-*d*]pyrimidines (**5**, **6**, **14**), and pyrazolo[3,4-*d*][1,2,3]triazine (**13**) were reported. Structure activity correlation of the obtained results revealed that the pyrazole derivatives **2**, **8**, **10**, and pyrazolo[3,4-*d*][1,2,3] triazine (**13**) showed the most significant antibacterial activities than the pyrazolo[3,4-*d*]pyrimidine derivatives **5**, **6**, and **14**. On the other hand, replacement of the hydroxyl moiety in the non-acetylated sugar **10** by acetyl group in **11** led to decrease the antibacterial potency.

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 Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), 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 part per million; (δ 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).

(6-Phenyl-pyridazin-3-yl)-hydrazine (1). It was prepared according to a reported method [16].

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid ethyl ester (2). To a solution of compound **1**, 1.86 g (0.01 mol) in 30 mL ethanol, 0.01 mol of ethyl(ethoxymethylene) cyanoacetate 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** Yield: (2.96 g, 95.79%,); mp 190–191 °C. IR spectrum (KBr, ν, cm^{-1}): 3443, 3330 (NH_2) and 1678 (CO); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.30 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 4.20 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 7.50–7.65 (m, 3H, Ar–H), 7.75 (s, 2H, NH_2, D_2O exchangeable), 7.90 (s, 1H, pyrazole-H), 8.10–8.25 (m, 3H, 2Ar–H + pyridazine-H), and 8.50 (d, J = 9.0 Hz, 1H, pyridazine-H); ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 15.2 (CH_3), 60.5 (OCH_2), 101 (C4), 134 (C3), 151 (C5), 160 (C=O), 120–156 (C-phenyl and C-pyridazine).

MS, *m/z* (%): 309 (M^+, 100), 281 (3.94), 263 (70.88), 237 (14.19), 210 (14.71), 197 (69.55), 185 (1.20), 171 (4.56), 156 (13.73), 140 (21.19), 115 (33.45), 104 (22.91), 77 (18.53). *Anal*. Calcd. for $C_{16}H_{15}N_5O_2$ (309.32): C, 62.12; H, 4.88; N, 22.64. Found: C, 61.98; H, 4.99; N, 22.68.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid hydrazide (3). Compound **2**, 3.09 g (0.01 mol), was heated in 50 mL hydrazine hydrate (99%) for 6 h. After cooling, the precipitated material was filtered off, washed several times with water, dried, and recrystallized from dioxane to give compound **3** (2.74 g, 92.88%); mp 297–298 °C. IR spectrum (KBr, ν, cm^{-1}): 3430, 3410, 3260 (NH_2, NH), and 1654 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 4.30 (s, 2H, NH_2, D_2O exchangeable), 7.50–7.70 (m, 5H, 3Ar−H + NH_2, exchangeable with D_2O), 8.05 (s, 1H, pyrazole-H), 8.10–8.25 (m, 3H, 2Ar–H + pyridazine-H), 8.43 (d, J = 8.5 Hz, 1H, pyridazine-H), and 9.15 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 295 (M^+, 33.06), 264 (100), 236 (1.17), 197 (3.39), 171 (1.83), 155 (4.76), 140 (2.17), 115 (15.26), 104 (4.13), 77 (12.29). *Anal*. Calcd. for $C_{14}H_{13}N_7O$ (295.29): C, 56.94; H, 4.43; N, 33.20. Found: C, 56.97; H, 4.34; N, 33.25.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carbonylazide (4). To a solution of compound **3**, 2.95 g (0.01 mol) in 10 mL glacial acetic acid at 5–10 °C, 3.5 mL chilled sodium nitrite solution 10% (0.01 mol), was added dropwise during 5 min with stirring. The reaction mixture was allowed to stand at room temperature for 1 h and then diluted with 25 mL water. The formed precipitate was filtered off and recrystallized from dioxane to give compound **4** (2.99 g, 97.71%); mp 194–196 °C. IR spectrum (KBr, ν, cm^{-1}): 3444, 3313 (NH_2), 2152 (N_3), and 1662 (C=O); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.50–7.65 (m, 3H, Ar–H), 7.90 (s, 1H, pyrazole-H), 8.05–8.25 (m, 4H, 2Ar–H + NH_2, D_2O exchangeable), and 8.30–8.50 (m, 2H, 2pyridazine-H); MS, *m/z* (%): 306 (M^+, 52.33), 278 (56.92), 264 (12.14), 250 (2.84), 223 (15.43), 209 (3.31), 197 (100), 168 (10.92), 155 (12.71), 140 (33.01), 115 (58.97), 104 (24.65), 77 (24.10). *Anal*. Calcd. for $C_{14}H_{10}N_8O$ (306.27): C, 54.89; H, 3.29; N, 36.58. Found: C, 54.64; H, 3.39; N, 36.74.

***N*-[4-oxo-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo[3,4-*d*]pyrimidin-5-yl]formamidic acid ethyl ester (5)**. Compound **3**, 2.95 g (0.01 mol) was refluxed in 40 mL triethyl orthoformate for 12 h. The formed precipitate on cooling was filtered off and recrystallized from dioxane to give compound **5** (3.33 g, 92.24%); mp 223–224 °C. IR spectrum (KBr, ν, cm^{-1}): 1700 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 1.36 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 4.37 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 7.56–7.58 (m, 3H, Ar–H), 8.17–8.20 (m, 2H, Ar–H), 8.31 (d, J = 9.3 Hz, 1H, pyridazine-H), 8.46–8.51 (m, 3H, pyrazole-H + pyridazine-H + N=CH), and 8.54 (s, 1H, pyrimidine-H). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 15 (CH_3), 69.5 (OCH_2), 108 (C3a), 135 (C3), 137 (C7a), 160 (C6), 168 (C=O), 119–154 (C-phenyl + C-pyridazine + C=N). MS, *m/z* (%): 361 (M^+, 72.71), 332 (24.90), 316 (13.04), 305 (34.44), 290 (100), 261 (30.50), 248 (17.17), 235 (12.30), 221 (10.74), 206 (12.11), 197 (14.35), 155 (12.22), 140 (12.64), 115 (20.95), 102 (15.35), 77 (17.73). *Anal*. Calcd. for $C_{18}H_{15}N_7O_2$ (361.35): C, 59.82; H, 4.18; N, 27.13. Found: C, 59.59; H, 4.06; N, 27.39.

***N*-[6-Methyl-4-oxo-1-(6-phenyl-pyridazin-3-yl)-1,4-dihydro-pyrazolo[3,4-*d*]pyrimidin-5-yl]acetamide (6)**. A mixture of compound **3**, 2.95 g (0.01 mol) in glacial acetic acid (20 mL) and acetic anhydride (20 mL) was refluxed for 12 h. The solution was cooled and poured into water and the formed precipitate was filtered off, washed with water, dried, and recrystallized from ethanol to give **6** (2.97 g, 82.27%); mp 165–167 °C. IR spectrum (KBr, ν, cm^{-1}): 3210 (NH), 1710 (CO) and 1685 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.11 (s, 3H, $COCH_3$), 2.43 (s, 3H, CH_3), 7.57–7.59 (m, 3H, Ar–H), 8.19–8.22 (m, 2H, Ar–H), 8.32 (d, J = 9.0 Hz, 1H, pyridazine-H), 8.49–8.52 (m, 2H, pyrazole-H + pyridazine-H), and 11.20 (s, 1H, NH, D_2O exchangeable). MS, *m/z* (%): 361 (M^+, 100), 346 (4.94), 319 (94.54), 289 (10.49), 262 (95.51), 221 (14.94), 197 (18.80), 139 (13.85), 115 (21.44), 101 (20.14), 91 (4.06), 77 (21.14). *Anal*. Calcd. for $C_{18}H_{15}N_7O_2$ (361.35): C, 59.82; H, 4.18; N, 27.13. Found: C, 60.03; H, 4.01; N, 26.91.

[5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazol-4-yl]-(3,5-dimethylpyrazol-1-yl)-methanone (7). A solution of compound **3**, 2.95 g (0.01 mol) in 20 mL acetylacetone was refluxed for 10 h. The solvent was then removed in vacuo and the remaining oily product was washed several times with dimethyl ether, dried, and recrystallized from chloroform to give compound **7** (3.05 g, 84.95%); mp 261–262 °C. IR spectrum (KBr, ν, cm^{-1}): 3444, 3318 (NH_2), and 1657 (C=O); ^1H NMR spectrum (DMSO-d_6, δ ppm): 2.25 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.20 (s, 1H, pyrazol-H), 7.50–7.65 (m, 3H, Ar–H), 8.10–8.40 (m, 5H, 2Ar–H + NH_2, D_2O exchangeable + pyridazine-H), 8.50 (d, J = 9.0 Hz, 1H, pyridazine-H), and 8.60 (s, 1H, pyrazole-H). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 10.5 (CH_3), 13.2 (CH_3), 100 (C4), 105 (C4′), 133 (C3), 146 (C3′), 147 (C5′) 153 (C5), 181 (C=O), 118–157 (C-phenyl and C-pyridazine). MS, *m/z* (%): 359 (M^+, 61.00), 334 (2.33), 319 (3.41), 264 (100), 197 (34.9), 179 (5.96), 155 (7.15), 140 (5.5), 115 (20.13), 104 (10.29), 77 (8.81). *Anal*. Calcd. for $C_{19}H_{17}N_7O$ (359.38): C, 63.49; H, 4.76; N, 27.28. Found: C, 63.24; H, 5.04; N, 27.22.

[5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazol-4-yl]-(5-amino-4-cyano-pyrazol-1-yl)-methanone (8). To a solution of compound **3**, 1.47 g (0.005 mol) in 20 mL dimethyl formamide, (0.005 mol) ethyl(ethoxymethylene)cyanoacetate was added, then the reaction mixture was refluxed for 3 h. The product that separated on cooling was filtered off and recrystallized from dioxane to give compound **8** (1.78 g, 95.96%,); mp 316–318 °C. IR spectrum (KBr, ν, cm^{-1}): 3419–3299 (2 NH_2), 2227 (CN), and 1660 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.45–7.80 (m, 5H, 3Ar–H + NH_2, D_2O exchangeable), 7.90–8.05 (m, 2H, Ar–H), 8.10–8.30 (m, 3H, pyrazole-H + NH_2, D_2O exchangeable), and 8.35–8.60 (m, 3H, 2pyridazine-H + pyrazole-H); MS, *m/z* (%): 371 (M^+, 25.10), 305 (1.18), 264 (100), 197 (2.90), 155 (1.40), 140 (3.01), 115 (5.76), 104 (1.45), 77 (1.60). *Anal*. Calcd. for $C_{18}H_{13}N_9O$ (371.34): C, 58.21; H, 3.52; N, 33.94. Found: C, 58.27; H, 3.24; N, 34.11.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid (4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydro-isoindol-2-yl)-amide (9). A mixture of compound **3**, 2.95 g (0.01 mol) and tetrachlorophthalic anhydride (2.85 g, 0.01 mol) in glacial acetic acid (50 mL) was refluxed for 3 h. The formed precipitate was filtered on hot, dried, and recrystallized from dioxane to give compound **9** (5.57 g, 98.93%); mp 340–341 °C. IR spectrum (KBr, ν, cm^{-1}): 3442, 3360 (NH_2, NH), 1799, 1743 (anhydride C=O), and 1647 (amide C=O); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.50–7.80 (m, 5H, 3Ar–H + NH_2, D_2O exchangeable), 8.05 (s, 1H, pyrazole-H), 8.15–8.40 (m, 2H, Ar–H), 8.50–8.60 (m, 2H, 2pyridazine-H), and 12.60 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 99 (C4), 133 (C3), 151 (C5), 166 (C=O) and 167.5 (C=O), 120–132 (C-phenyl and C-pyridazine). *Anal.* Calcd. for $C_{22}H_{11}Cl_4N_7O_3$ (563.19): C, 46.91; H, 1.97; N, 17.41. Found: C, 46.73; H, 2.20; N, 17.40.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid (2,3,4,5,6-pentahydroxy-hexylidine)-hydrazide (10). A mixture of 2.95 g (0.01 mol) of compound **3**, D-glucose (1.8 g, 0.01 mol) in dry dimethylformamide (50 mL) and a catalytic amount of acetic acid were heated at 80 °C for 1 h. The formed precipitate was filtered on hot, washed with ethanol several times and dried to give compound **10** (4.43 g, 96.93% yield); mp 248–

249 °C. IR spectrum (KBr, ν, cm^{-1}): 3442–3320 (broad, OH, NH, NH_2), and 1689 (C═O); ^1H NMR spectrum (DMSO-d_6, δ ppm): 3.25–3.60 (the protons of the alditol congregated with the water signal) [18], 3.65–3.90 (m, 2H, CH_2OH), 4.20–4.35 (m, 1H, OH, D_2O exchangeable), 4.90–5.05 (m, 2H, OH, D_2O exchangeable), 5.30 (d, J = 5.4 Hz, 1H, OH, D_2O exchangeable), 5.80 (d, J = 4.7 Hz, 1H, OH, D_2O exchangeable), 7.50–7.65 (m, 3H, Ar–H), 7.75 (s, 2H, NH_2, D_2O exchangeable), 8.10–8.30 (m, 5H, 2Ar–H + pyrazole-H + pyridazine-H + N═CH), 8.05 (d, J = 9.0 Hz, 1H, pyridazine-H), and 9.60 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 61.2–73.2 (C-alditol), 100 (C4), 134 (C3), 151 (C5), 159 (C═O), 126.3–162.9 (C-phenyl + C-pyridazine + N═*C*H). *Anal*. Calcd. for $C_{20}H_{23}N_7O_6$ (457.44): C, 52.50; H, 5.06; N, 21.43. Found: C, 52.67; H, 4.77; N, 21.48.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid(2,3,4,5,6-penta-*O*-acetyl-hexylidine)-hydrazide (11). A solution of compound **10**, 2.29 g (0.005 mol) in a mixture of acetic anhydride (10 mL), acetic acid (10 mL), and pyridine (10 mL) was stirred for 8 h at room temperature. The reaction mixture was poured onto water and the precipitate was collected by filtration, washed with water, dried, and purified by using TLC plate to give compound **11** (1.64 g, 49.17%) as a major product; mp 139–141 °C. IR spectrum (KBr, ν, cm^{-1}): 3400, 3325 (NH, NH_2), 1711, and 1689 (2C═O); ^1H NMR spectrum (DMSO-d_6, δ ppm): 1.95–2.06 (m, 15H, 5CH_3CO), 3.20–3.59 (alditol protons congregated with the water signal), 7.55–7.57 (m, 3H, Ar–H), 8.14 (s, 2H, NH_2, D_2O exchangeable), 8.16–8.22 (m, 5H, 2 Ar–H + pyrazole-H + pyridazine-H + N═CH), 8.44 (d, J = 9.3 Hz, 1H, pyridazine-H), and 11.20 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 20.5–21.1 (5CH_3), 61.3–71.8 (C-alditol), 100 (C4), 134 (C3), 151 (C5), 160 (C═O), 168.9–172.2 (5 C═O), 126.3–162.9 (C-phenyl + C-pyridazine + N═*C*H). *Anal*. Calcd. for $C_{30}H_{33}N_7O_{11}$ (667.62): C, 53.97; H, 4.98; N, 14.69. Found: C, 53.90; H, 5.01; N, 14.72.

5-Amino-1-(6-phenyl-pyridazin-3-yl)-1*H*-pyrazole-4-carboxylic acid benzylidene-hydrazide (12). A mixture of compound **3**, 2.95 g (0.01 mol), benzaldehyde (0.01 mol) and few drops of piperidine in 50 mL dioxane was refluxed for 3 h. The reaction mixture was kept at room temperature overnight, then the separated solid was filtered off, dried, and recrystallized from dioxane to give compound **12** (3.14 g, 81.98%); mp 291–292 °C. IR spectrum (KBr, ν, cm^{-1}): 3423, 3313 (NH, NH_2), and 1680 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.40–7.75 (m, 10H, Ar–H), 8.05 (s, 2H, NH_2, D_2O exchangeable), 8.10–8.30 (m, 3H, pyrazole-H + N═CH + pyridazine-H), 8.47 (d, J = 8.0 Hz, 1H, pyridazine-H), and 11.40 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 383 (M^+, 42.36), 351 (3.73), 306 (1.57), 280 (27.11), 263 (100), 237 (4.48), 197 (10.42), 171 (3.99), 155 (14.00), 140 (14.89), 115 (41.82), 104 (12.55), 89 (15.54), 77 (21.05). *Anal*. Calcd. for $C_{21}H_{17}N_7O$ (383.40): C, 65.78; H, 4.47; N, 25.57. Found: C, 65.69; H, 4.49; N, 25.46.

***5*-(Benzylidene-amino)-1-(6-phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*][1,2,3]triazin-4-one (13)**. To an ice cooled mixture of compound **12**, 1.91 g (0.005 mol) in 30 mL of acetic acid, sodium nitrite solution (0.5 g in 2 mL of water) was added dropwise during 15 min with stirring, and then the reaction mixture was stirring at room temperature for an additional 1 h. The solid product was filtered off and recrystallized from dioxane to give compound **13** (1.54 g, 78.17%); mp 277–279 °C. IR spectrum (KBr, ν, cm^{-1}): 1737 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.56–7.66 (m, 6H, Ar–H), 8.02–8.05 (m, 2H, Ar–H), 8.22–8.24 (m, 2H, Ar–H), 8.39 (d, J = 9.3 Hz, 1H, pyridazine-H), 8.59 (d, J = 9.3 Hz, 1H, pyridazine-H), 8.86 (s, 1H, pyrazole-H), and 9.21 (s, 1H, N═CH). ^{13}C NMR spectrum (DMSO-d_6, δ ppm): 108 (C3a), 135 (C3), 137 (C7a), 168 (C═O), 118–162.9 (C-phenyl + C-pyridazine + N═*C*H). MS, *m/z* (%): 394 (M^+, 2.10), 366 (0.95), 338 (8.16), 309 (16.62), 282 (9.01), 265 (11.92), 249 (49.69), 221 (28.21), 206 (16.39), 164 (5.90), 140 (7.87), 115 (18.29), 102 (47.23), 90 (100), 77 (19.25). *Anal*. Calcd. for $C_{21}H_{14}N_8O$ (394.38): C, 63.95; H, 3.58; N, 28.41. Found: C, 64.07; H, 3.62; N, 28.21.

5-(Benzylidene-amino)-1-(6-phenyl-pyridazin-3-yl)-1,5-dihydro-pyrazolo[3,4-*d*]pyrimidin-4-one (14). A mixture of compound **12**, 1.91 g (0.005 mol) in 50 mL of triethyl orthoformate was refluxed for 8 h. The product that separated on cooling was filtered off and recrystallized from dimethylformamide to give compound **14** (1.85 g, 94.14%), mp 303–304 °C. IR spectrum (KBr, ν, cm^{-1}): 1698 (CO); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.50-7.70 (m, 6H, Ar–H), 8.10–8.25 (m, 4H, Ar–H), 8.40–8.60 (m, 2H, pyrazole-H + pyridazine-H), 8.80–9.00 (m, 2H, pyridazine-H + N═CH), and 9.30 (s, 1H, pyrimidine-H); MS, *m/z* (%): 393 (M^+, 2.48), 290 (100), 261 (20.37), 248 (4.45), 235 (6.34), 206 (7.15), 179 (2.14), 155 (2.35), 140 (4.92), 115 (7.09), 102 (26.31), 77 (9.28). *Anal*. Calcd. for $C_{22}H_{15}N_7O$ (393.39): C, 67.16; H, 3.84; N, 24.92. Found: C, 66.99; H, 3.80; N, 25.09.

4-(5-phenyl-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazole-3-ylamine (15). A solution of compound **12**, 1.91 g (0.005 mol) in 1,4-dichlorobenzene (20 mL) was refluxed for 1 h. The product that separated on cooling was filtered off and recrystallized from dimethyl formamide to give compound **15** (1.76 g, 91.90% yield); mp 245–247 °C. IR spectrum (KBr, ν, cm^{-1}): 3425, 3315 (NH_2) and 3234 (NH); ^1H NMR spectrum (DMSO-d_6, δ ppm): 5.90 (s, 1H, oxadiazole-H), 7.30–7.90 (m, 8H, 6Ar–H + NH_2, D_2O exchangeable), 8.00 (s, 1H, pyrazole-H), 8.10–8.30 (m, 4H, Ar–H), 8.45–8.55 (m, 2H, 2pyridazine-H), and 10.20 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 383 (M^+, 3.57), 280 (20.29), 264 (100), 237 (5.13), 197 (6.11), 155 (6.02), 140 (4.22), 115 (21.47), 104 (7.28), 77 (10.86). *Anal*. Calcd. for $C_{21}H_{17}N_7O$ (383.40): C, 65.78; H, 4.47; N, 25.57. Found: C, 66.00; H, 4.50; N, 25.30.

4-(5-phenyl-[1,3,4]oxadiazol-2-yl)-2-(6-phenyl-pyridazin-3-yl)-2*H*-pyrazole-3-ylamine (16). **Method A**: To a suspension of compound **12**, 1.91 g (0.005 mol) and anhydrous sodium acetate (0.005 mol) in glacial acetic acid (20 mL); bromine (0.005 mol) in glacial acetic acid (2 mL) was added with stirring. The mixture was refluxed for 1 h, then poured onto water and the precipitated solid was filtered off, washed with water, dried and recrystallized from dimethylformamide to give compound **16** (1.83 g, 96.06%); mp 304–306 °C.

Method B: To a suspension of 1.92 g (0.005 mol) of compound **15** in 20 mL glacial acetic acid, bromine (0.005 mol) in 2 mL glacial acetic acid was added with stirring. The mixture was refluxed for 1 h then poured onto water and the precipitated solid was filtered off, washed with water, dried, and recrystallized from dimethylformamide to give a compound (1.15 g, 60.36%) identical in all aspects with compound **16** (mp, mixed mp, and TLC), mp 304–306 °C. IR spectrum (KBr, ν, cm^{-1}): 3436, 3316 (NH_2); ^1H NMR spectrum (DMSO-d_6, δ ppm): 7.55–7.70 (m, 6H, Ar–H), 7.75 (s, 2H, NH_2, D_2O exchangeable), 8.10–8.40 (m, 6H, 4Ar–H + pyrazole-H + pyridazine-H), and 8.55 (d, J = 9.0 Hz, 1H, pyridazine-H); MS, *m/z* (%): 381 (M^+, 100), 353 (4.65), 340 (2.82), 304 (14.27), 263 (9.56), 212 (6.96), 196 (9.34), 154 (5.81), 143 (5.83), 115 (5.63), 105 (8.16), 88 (13.29), 77

(4.07). *Anal*. Calcd. for $C_{21}H_{15}N_7O$ (381.38): C, 66.13; H, 3.96; N, 25.70. Found: C, 65.92; H, 4.01; N, 25.76.

BIOLOGICAL EVALUATION

The *in vitro* antimicrobial activity of the synthesized compounds was tested against several pathogenic representatives: *Escherichia coli, Bacillus subtilis, Candida albicans*, and *Aspergillus niger*. All micro-organisms used were obtained from Chemistry of Natural and Microbial Products Department, National Research Centre, Cairo, Egypt. Disk diffusion sensitivity test was carried out in the manner identical to that of Bauer *et al*. [19]. Media for disk sensitivity tests were nutrient agar and Muller–Hinton agar, purchased from Difco, (USA). The non-sterile powder of the tested compounds was dissolved in sterile DMSO to yield $2\,\mu g\,mL^{-1}$ passed through 0.2 μm membrane filter (Millipore Corp., USA). The filtrates were dispensed as 2 mL samples into sterile, small screw-capped vials and kept stored at −15 °C. DMSO as a solvent showed no inhibition zones. The results were compared with Streptomycin as a reference drug.

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