Full Paper

Synthesis, Isomerization, and Antimicrobial Evaluation of Some Pyrazolopyranotriazolopyrimidine Derivatives

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6-Amino-5-imino-pyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine derivative **4** and pyrazolo-[4′,3′:5,6]pyrano[2,3-*d*]pyrimidin-5-ylhydrazine derivative **5** were prepared starting from 6-amino-3-methyl-4-(*p*-nitrophenyl)-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile **1**. The synthesis and structure characterization of 9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **7** and **9** and their isomerization to 9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **6** and **8**, respectively, under different suitable reaction conditions are reported. Moreover, the synthesis of 9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine derivative **14** and N^9-acyclic nucleoside **15** are described. Some of the prepared products showed potent antimicrobial activity.

Keywords: Acyclic nucleosides / Antimicrobial activities / Pyranopyrazoles / Pyrazolopyranopyrimidines / Pyrazolopyranotriazolopyrimidines

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Introduction

The synthesis of triazolopyrimidines fused to other heterocyclic moieties has been described by many investigators; the compounds proved to have pronounced biological activities [1 – 5]. Previous observations revealed that the [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives can isomerize under different suitable reaction conditions to the thermodynamically more stable [1,2,4]triazolo[1,5-*c*]pyrimidines [6 – 11]. This isomerization was reported earlier by Miller and Rose [12, 13], when they treated [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives with acid, base, or thermally. Nevertheless, this pattern of isomerization appears to have been overlooked by a number of workers [4, 5, 14, 15]. In continuation of our previous work on pyrano[2,3-*c*]pyrazoles [16], fused pyrimidines [11, 17 – 19] and pyrazolopyranopyrimidines [20], we aimed to synthesize pyrazolopyranotriazolo[4,3-*c*]pyrimidines and pyrazolopyranotriazolo[1,5-*c*]pyrimidines not only to study their isomerization, but also to obtain new compounds which are expected to possess notable chemical and biological activities.

Results and discussion

Chemistry

5-Amino-2,4-dihydro-3-methyl-4-(*p*-nitrophenyl)pyrano-[2,3-*c*]pyrazole-5-carbonitrile **1** [16, 20], as the key compound for this study and for further syntheses of other fused heterocyclic compounds, was heated at reflux temperature with an equimolar amount of triethyl orthoacetate in the presence of acetic anhydride to give a major product which could be assigned the structure of ethyl *N*-[2-acetyl-5-cyano-3-methyl-4-(*p*-nitrophenyl)-2,4-dihydropyrano[2,3-*c*]pyrazol-6-yl]ethanimidate **2** (Scheme 1). Inspection of the ^{1}H-NMR spectra of the reaction product **2** revealed the absence of the signal characteristic for the NH protons of the pyrazole moiety. The acetylation was assigned to take place at N^2 position which is in agreement with the acetylation reported in one of our recent publications [20]. Deacetylation of compound **2** was car-

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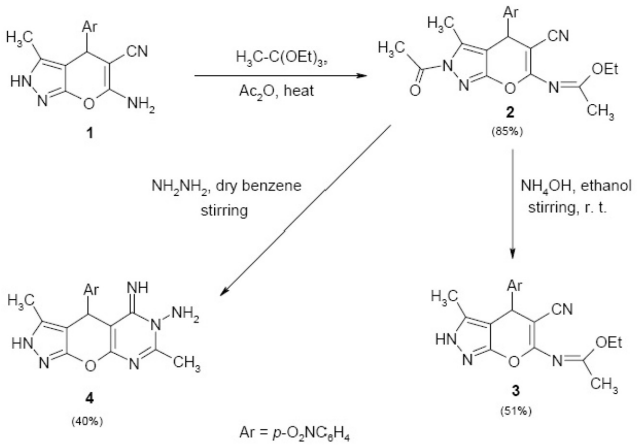


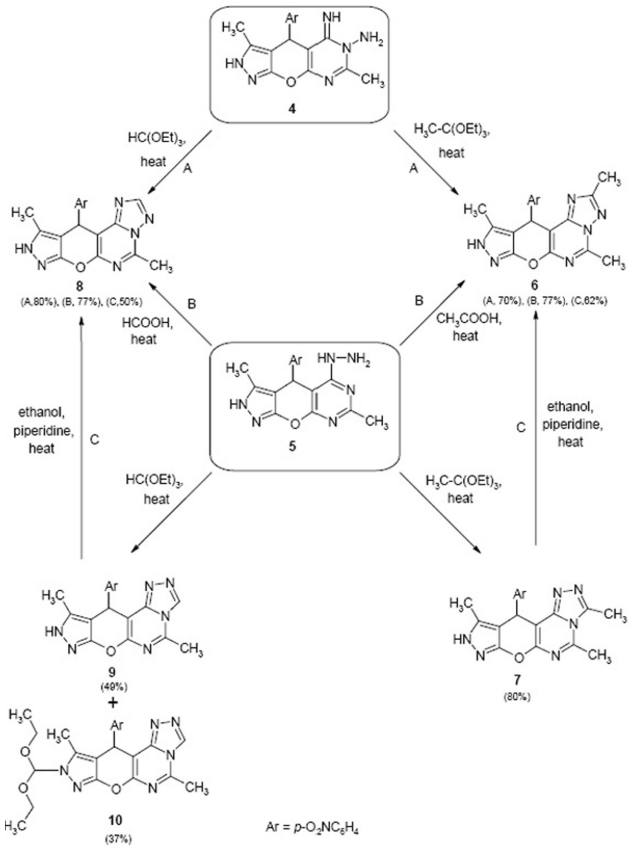
Scheme 1. Deacetylation of compound **2**.

ried out under mild conditions by treatment with ammonium hydroxide solution to give ethyl *N*-[5-cyano-3-methyl-4-(*p*-nitrophenyl)-2,4-dihydropyrano[2,3-*c*]pyrazol-6-yl]ethanimidate **3** (Scheme 1).

On the other hand, when a solution of compound **2**, in dry benzene, was stirred with hydrazine hydrate, it afforded 6-amino-3,7-dimethyl-5-imino-4-(*p*-nitrophenyl)-2,4,5,6-tetrahydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine **4** (Scheme 1). The ^{1}H-NMR spectrum of the latter compound revealed the absence of signals of the acetyl protons and the ethoxy group protons and showed signals for NH_2 and 2 times NH (D_2O exchangeable).

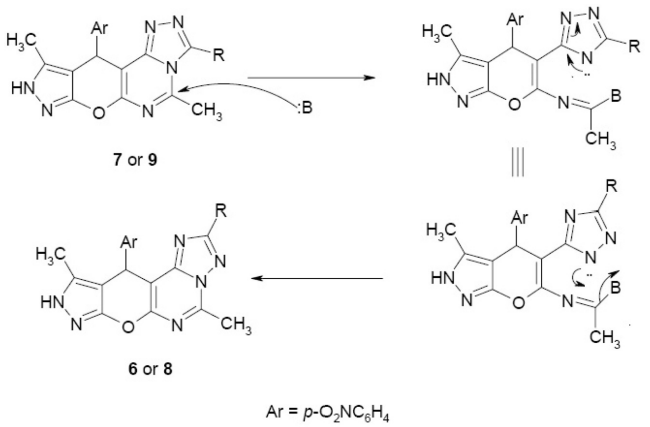
Heating of compound **4** or **5** [20] with triethyl orthoacetate at reflux temperature, gave 2,5,10-trimethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidine **6** or 3,5,10-trimethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6] pyrano[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine **7**, respectively (Scheme 2). It was noticed that the two triazolopyrimidine derivatives **6** and **7** showed no appreciable difference in the fragmentation pattern under electron impact (see Experimental), however, the ^{1}H-NMR spectra of triazolo[4,3-*c*]pyrimidine derivative **7** revealed that the methyl protons (C^3-CH_3 and C^5-CH_3), respectively, appeared at a more downfield chemical shift when compared with that of the [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **6** (Table 1); these data are in agreement with the reported results of related compounds [6, 7, 11].

This confirmed that the product obtained from the reaction with hydrazino derivative **5** differs from those obtained from the reaction with the imino derivative **4**. However, when compound **5** was heated under reflux temperature in acetic acid, it afforded compound **6** probably via the intermediacy of its isomer [1,2,4]triazolo[4,3-*c*]pyrimidine **7** which was not isolated in this reaction, but



Scheme 2. Synthesis route of compound **4**–**10**.

underwent a Dimroth-type rearrangement [9, 21] under the conditions of the reaction. To prove this assumption, compound **7** was converted into its corresponding [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **6**, by heating in presence of a base, which presumably involves a sequence of ring opening and ring closure reactions as depicted in Scheme 3. Likewise, when compound **4** was refluxed with triethyl orthoformate, it gave 5,10-dimethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4] triazolo[1,5-*c*]pyrimidine **8** (Scheme 2). While heating of compound **5** with triethyl orthoformate, gave two products to which the structures of: 5,10-dimethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′: 5,6] pyrano[3,2-*e*] [1,2,4] triazolo[4,3-*c*]pyrimidine **9**, as a major product, and 9,9-diethoxymethyl-5,10-dimethyl-11-(*p*-nitrophenyl)-9,11-dihydro-pyrazole[4′,3′:5,6] pyrano[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine **10**, isolated as a side product in 37% yield, could be assigned (Scheme 2). Triethyl orthoformate is considered as an alkylating agent for the NH of the pyrazole moiety, and compound **10** can be considered as a new pyrazole N^9-acyclonucleoside. The triazolopyrimidine derivatives **8**, **9**, and **10** showed differences in the ^{1}H-NMR spectra, where the triazolo[4,3-*c*]pyrimidine deriv-



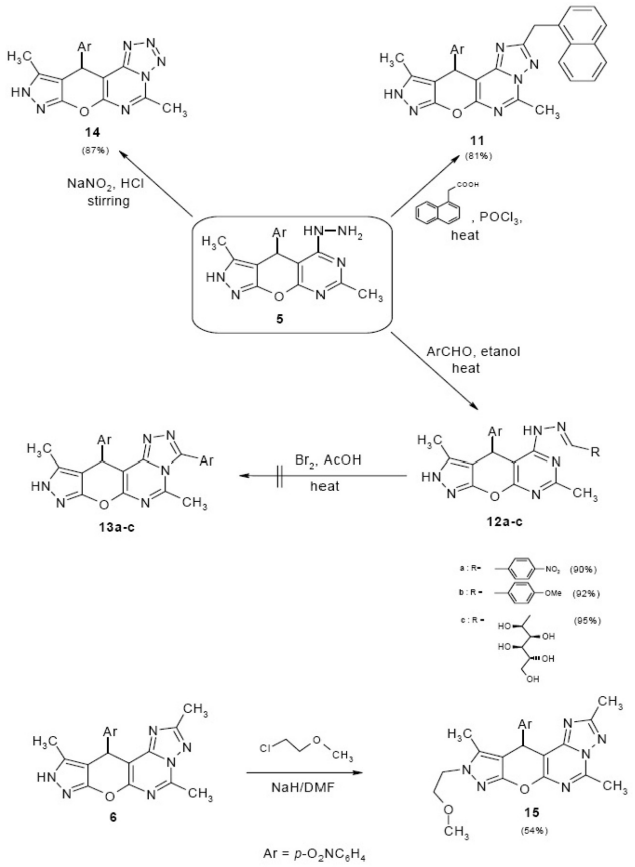
Scheme 3. Conversion of compounds **8** and **9**.

Table 1. Correlations of ^{1}H-NMR spectra.

Compd. N°	δ C_5-CH_3	δ C_3-H	d C_2-H	δ C_3-CH_3	δ C_2-CH_3
6	2.36	–	–	–	2.81
7	2.82	–	–	2.93	–
8	2.79	–	8.41	–	–
9	2.85	9.45	–	–	–
10	2.83	9.34	–	–	–
11	2.38	–	–	–	–
14	2.99	–	–	–	–
15	2.27	–	–	–	2.73

atives **9** and **10** revealed that the protons (C^3-H and C^5-CH_3), respectively, appeared at a more downfield chemical shift when compared with that of the [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **8** (Table 1). Also, when compound **5** was refluxed with formic acid, it afforded compound **8** probably via the intermediacy of its isomer [1,2,4]triazolo[4,3-*c*]pyrimidine **9;** and to prove this assumption, compound **9** was converted into its corresponding [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **8**, by heating in piperidine and ethanol (Scheme 3).

Similarly, when the hydrazino derivative **5** was refluxed with 1-naphthylacetic acid in the presence of phosphorus oxychloride, it afforded the polycyclic 5,10-dimethyl-3-naphthalen-1-ylmethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **11** probably via a Dimroth-type rearrangement of the [1,2,4]triazolo[4,3-*c*]pyrimidine intermediate under the acidic conditions of the reaction [6, 7]. That was judged from the ^{1}H-NMR signal of the methyl proton (C^5-CH_3) which appeared at d 2.38 ppm; if the [1,2,4]triazolo[4,3-*c*] isomer was the product, the C^5-methyl group would have been expected to be more deshielded and to appear at a more downfield position (Table 1); these data are in agreement with reported results of related compounds [6, 7, 11].



Scheme 4. Synthesis route of compound **11 – 15**.

Condensation of compound **5** with *p*-nitrobenzaldehyde, *p*-methoxybenzaldehyde in the presence of a few drops of piperidine and also with aldohexose [22, 23], namely D-glucose, in the presence of a catalytic amount of glacial acetic acid took place by heating under reflux in ethanol, where the corresponding hydrazones **12a-c** were produced. Attemps to cyclize the latter compounds to their corresponding [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives by refluxing with bromine in glacial acetic acid failed.

Heterocyclic azides, especially azidomethines, can exist in equilibrium with their tetrazolo tautomers and this equilibrium is affected by many factors: pH, temperature, the nature of the substituents around the (C=N), and the used solvent [24 – 26]. This equilibrium can be shifted in either direction by controlling these factors, and the IR spectroscopy is helpful in revealing which form is predominant, since the azido structure can show a characteristic band in the region ν 2100 – 2200 cm^{-1}.

Thus, nitrosation of the hydrazino group of compound **5**, afforded 5,10-dimethyl-11-(*p*-nitrophenyl)-9,11-dihy-

dropyrazolo[4′,3′:5,6]pyrano[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine **14**. Analytical and spectral data are in agreement with the proposed structure (see Experimental). In particular, the IR spectrum of compound **14** did not show absorption frequency indicative for the azido group. The formation of the tetrazolo structure in compound **14** may be caused by the effect of an electron-donating group (CH_3), which stabilizes the tetrazolo structure.

The N^9-acyclic nucleosides of pyrazolo[4′,3′:5,6]pyrano[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives are still not known (to the best of our knowledge) in the literature. In continuation of our previous work in preparing various cyclic and acyclic nucleosides of different heterocyclic compounds[17, 18, 20, 22, 27], in this report, we described the synthesis of N^9-acyclic nucleoside related to pyrazolo[4′,3′:5,6]pyrano[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives by treating the sodium salt of compound **7** (generated *in situ*, see Experimental) with 2-chloroethyl methyl ether to afford the corresponding N^9-acyclic nucleoside: 9-(2-methoxyethyl)-2,5,10-trimethyl-11-(*p*-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **15** (Scheme 4). The structural assignment and site of nucleosidation of compound **15** was assigned on the basis of the elemental and spectral data (see Experimental).

Biological evaluation

The antimicrobial activity of some newly synthesized compounds **1**, **4**, **5**, **6**, **7**, **8**, **9**, **11**, **12c**, and **14** were tested and the results are shown in Table 2. Evaluation of the new compounds established that compounds **5**, **7**, **9**, **12c**, and **14** were slightly active against *gram*-positive and *gram*-negative bacteria. On the other hand, it was found that compounds **7** and **9** revealed more effective activity against yeast than the other tested compounds while compounds **5**, **6**, **7**, **9**, **12c**, and **14** revealed a slight to strong antifungal activities. In particular, compound **7** revealed higher antifungal activity than that of the other tested compounds and the reference drug.

Table 2. Antimicrobial activity of some synthesized compounds[a].

	Disc diffusion test (mm) Microorganisms			
	Bacteria			
Compound	*Gram*-negative *Escherichia coli*	*Gram*-positive *Bacillus subtillis*	Yeast *Candida albicans*	Fungi *Aspergillus niger*
Streptomycin[b]	+++	+++	+++	+
1	+	-	-	-
4	+	-	-	-
5	+	+	-	++
6	+	-	-	+
7	+	+	++	+++
8	+	-	-	-
9	+	+	+	++
11	+	-	-	-
12c	+	+	-	++
14	+	+	-	++

[a] γ = 2 μg/mL in DMSO.
[b] γ = 25 μg/mL in DMSO, (Lot. 30730, Bioanalyse Turkey).
+++ highly sensitive (14–16 mm).
++ fairly sensitive (12–14 mm).
+ slightly sensitive (10–12 mm); – not sensitive.

Conclusion

Synthesis and structure characterization of pyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **7** and **9** and their isomerization to pyrazolo[4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **6** and **8**, respectively, are reported. Moreover, pyrazolo[4′,3′:5,6]pyrano[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine derivative **14** was obtained. Structure-activity correlations of the obtained results revealed that the [1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **7** and **9** showed antifungal activity more effective than that of the isomeric [1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **6**, **8**, and **11**. On the other hand, the hydrazino, hydrazone, and tetrazolo[1,5-*c*]pyrimidine derivatives **5**, **12c**, and **14** revealed antifungal activity more effective than that of the amino imino derivative **4**.

We are grateful to Dr. H. M. Awad, Department of Natural and Microbial Products, National Research Centre, for his biological evaluation.

Experimental

Chemistry

All melting points are uncorrected and measured using Electrothermal IA 9100 apparatus, (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin Elmer, Norwalk, CT, USA), National Research Centre, Cairo, Egypt. ^{1}H-NMR and ^{13}C-NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on EI + Q1 MSLMR UPLR, National Research Centre, Cairo, Egypt. Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, National Research Centre, Cairo, Egypt and the results were within the accepted range (± 0.40) of the calculated values. Column chroma-

tography was performed on Silica gel 60 (particle size 0.06 – 0.20 mm; Merck, Darmstadt, Germany). Compounds **1** and **5** were prepared according to a reported method [16].

Ethyl-N-[2-acetyl-5-cyano-3-methyl-4-(p-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazol-6-yl]ethanimidate ***2***

A mixture of compound **1** (2.97 g, 10 mmol) in triethyl orthoacetate (5 mL) and acetic anhydride (5 mL) was refluxed for 5 h. The reaction mixture was evaporated under reduced pressure and the residue was filtered off, dried, and recrystallized from absolute ethanol to give compound **2**. Yield 85%, mp. 160–162°C. IR ν 2220 (CN) and 1720 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.28 (t, *J* = 9 Hz, 3H, OCH_2CH_3), 2.13 (s, 3H, C^3-CH_3), 2.15 (s, 3H, N=C-CH_3), 2.48 (s, 3H, O=C-CH_3), 4.24 (q, *J* = 9 Hz, 2H, OCH_2CH_3), 5.20 (s, 1H, pyran-H), 7.62 (d, *J* = 9 Hz, 2H, Ar-H), 8.24 (d, *J* = 9 Hz, 2H, Ar-H). MS *m/z* (%): 409 [M^+] (81.25). Anal. calcd. for $C_{20}H_{19}N_5O_5$: C 58.68, H 4.68, N 17.11. Found: C 58.61, H 4.63, N 17.23.

Ethyl-N-[5-cyano-3-methyl-4-(p-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazol-6-yl]ethanimidate ***3***

To a solution of compound **2** (2.45 g, 5 mmol) in absolute ethanol (20 mL), ammonium hydroxide solution (1 mL, 25%) was added with stirring at room temperature for 1 h. The formed precipitate was filtered off, dried, and recrystallized from absolute ethanol to give compound **3**. Yield 51%, mp. 238 – 240°C. IR ν 3279 (NH), 2215 (CN) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.28 (t, *J* = 9 Hz, 3H, OCH_2CH_3), 1.80 (s, 3H, C^3-CH_3), 2.12 (s, 3H, N=C-CH_3), 4.23 (q, *J* = 9 Hz, 2H, OCH_2CH_3), 5.08 (s, 1H, pyran-H), 7.56 (d, *J* = 9 Hz, 2H, Ar-H), 8.23 (d, *J* = 9 Hz, 2H, Ar-H), 12.50 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 367 [M^+] (61.04). Anal. calcd. for $C_{18}H_{17}N_5O_4$: C 58.85, H 4.66, N 19.06. Found: C 58.91, H 4.67, N 18.99.

6-Amino-3,7-dimethyl-5-imino-4-(p-nitrophenyl)-2,4,5,6-tetrahydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidine ***4***

To a solution of compound **2** (0.40 g, 1 mmol) in dry benzene (30 mL), hydrazine hydrate (4 mL, 99%) was added with stirring at 0°C for 1 h. The obtained product was filtered off, dried, and recrystallized from ethanol to give compound **4**. Yield 40%, mp. 282 – 284°C. IR ν 3240 – 3160 (NH_2, NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.93 (s, 3H, C^3-CH_3), 2.41 (s, 3H, C^7-CH_3), 5.21 (s, 1H, pyran-H), 5.67 (brs, 2H, NH_2, D_2O exchangeable), 6.45 (s, 1H, NH, D_2O exchangeable), 7.56 (d, *J* = 9 Hz, 2H, Ar-H), 8.12 (d, *J* = 8.4 Hz, 2H, Ar-H), 12.06 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 353 [M^+] (19.6). Anal. calcd. for $C_{16}H_{15}N_7O_3$: C 54.39, H 4.28, N 27.75. Found: C 54.45, H 4.33, N 27.64.

2,5,10-Trimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine ***6***

Method A: A mixture of compound **4** (0.18 g, 0.5 mmol) and triethyl orthoacetate (30 mL) was refluxed for 10 h. The reaction mixture was evaporated to dryness and the remaining solid was recrystallized from dioxane to give compound **6**. Yield 70%, mp. 364 – 365°C.

Method B: Compound **5** (3.53 g, 1 mmol) was heated under reflux temperature in acetic acid (30 mL) for 8 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give a product identical in all aspects with compound **6** obtained before. Yield 77%, mp. 364 – 365°C.

Method C: A solution of compound **7** (0.37 g, 1 mmol) in ethanol (20 mL) in the presence of a few drops of piperidine was heated under reflux temperature for 30 min. The solvent was removed under reduced pressure leaving a solid product which was recrystallized from dioxane to give a compound identical in all aspects with compound **6** obtained before. Yield 62%, mp. 364 – 366°C. IR ν 3225 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.90 (s, 3H, C^{10}-CH_3), 2.36 (s, 3H, C^5-CH_3), 2.81 (s, 3H, C^2-CH_3), 5.72 (s, 1H, pyran-H), 7.57 (d, *J* = 9 Hz, 2H, Ar-H), 8.11 (d, *J* = 8.4 Hz, 2H, Ar-H), 12.31 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 9.8 (C10-CH_3), 12.8 (C2-CH_3), 19.9 (C5-CH_3), 34.7 (C-11), 95.5 (C-11a), 97.3 (C-10a), 123, 129, 134.5, 136 (Ar-C), 144.5 (C-7a), 146.1 (C-10), 148.5 (C-11b), 149.5 (C-2), 150.5 (C-5), 151.2 (C-6a). MS *m/z* (%): 377 [M^+] (18.01). Anal. calcd. for $C_{18}H_{15}N_7O_3$: C 57.29, H 4.01, N 25.98. Found: C 57.40, H 3.98, N 25.90.

3,5,10-Trimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine ***7***

Compound **5** (3.53 g, 10 mmol) was heated under reflux temperature in triethyl orthoacetate (40 mL) for 5 h. The product which separated on cooling was filtered off, dried, and recrystallized from dioxane to give compound **7**. Yield 80%, mp. 323 – 325°C. IR ν 3228 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.96 (s, 3H, C^{10}-CH_3), 2.82 (s, 3H, C^5-CH_3), 2.93 (s, 3H, C^3-CH_3), 5.67 (s, 1H, pyran-H), 7.58 (d, *J* = 9 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.4 Hz, 2H, Ar-H), 12.24 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 9.8 (C10-CH_3), 13.6 (C3-CH_3), 21.8 (C5-CH_3), 34.8 (C-11), 95.9 (C-11a), 97.4 (C-10a), 123, 128, 129, 136 (Ar-C), 144.5 (C-7a), 146.2 (C-10), 149.1 (C-11b), 150.5 (C-3), 151.2 (C-5), 151.6 (C-6a). MS *m/z* (%): 377 [M^+] (29.98). Anal. calcd. for $C_{18}H_{15}N_7O_3$: C 57.29, H 4.01, N 25.98. Found: C 57.33, H 4.11, N 25.84.

5,10-Dimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine ***8***

Method A: A mixture of compound **4** (0.35 g, 1 mmol) and triethyl orthoformate (30 mL) was refluxed for 10 h. On cooling a precipitate formed which was filtered off, dried, and recrystallized from dioxane to give compound **8**. Yield 80%, mp. 357 – 359°C.

Method B: Compound **5** (3.53 g, 1 mmol) was heated under reflux temperature in formic acid (40 mL, 85%) for 10 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from dioxane to give a product identical in all aspects with compound **8** obtained before. Yield 77%, mp. 357 – 359°C.

Method C: A solution of compound **9** (0.39 g, 1 mmol) in ethanol (20 mL) in a presence of a few drops of piperidine was heated under reflux temperature for 1 h. The reaction mixture was evaporated to dryness and the remaining solid was recrystallized from dioxane to give a compound identical in all aspects with compound **8** obtained before. Yield 50%, mp. 357–359°C. IR ν 3224 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.96 (s, 3H, C^{10}-CH_3), 2.79 (s, 3H, C^5-CH_3), 5.77 (s, 1H, pyran-H), 7.58 (d, *J* = 9 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.41 (s, 1H, C^2-H), 12.34 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 9.8 (C10-CH_3), 19.8 (C5-CH_3), 35 (C-11), 95 (C-11a), 97.3 (C-10a), 123, 129, 135, 136 (Ar-C), 146 (C-7a), 147.5 (C-10), 148.3 (C-2), 150 (C-11b), 151.5 (C-5), 154.8 (C-6a). MS *m/z* (%): 363 [M^+] (19.33). Anal. calcd. for $C_{17}H_{13}N_7O_3$: C 56.20, H 3.61, N 26.99. Found: C 56.31, H 3.58, N 26.91.

*Synthesis of compounds **9** and **10***

Compound **5** (3.53 g, 10 mmol) was heated under reflux temperature in triethyl orthoformate (40 mL) for 5 h. The reaction mixture was kept at room temperature overnight, then the solvent was evaporated to dryness and the remaining solid was purified on TLC plate using chloroform: methanol (9 : 1) as an eluent to separate two products, **9** as a major product and **10** as a minor product.

*5,10-Dimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine **9***

Yield 49%, mp. 390 – 392°C. IR ν 3225 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.96 (s, 3H, C^{10}-CH_3), 2.85 (s, 3H, C^5-CH_3), 5.75(s, 1H, pyran-H), 7.60 (d, *J* = 9 Hz, 2H, Ar-H), 8.15 (d, *J* = 9 Hz, 2H, Ar-H), 9.45 (s, 1 H, C^3-H), 12.35 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 9.9 (C10-CH_3), 20.5 (C5-CH_3), 35 (C-11), 95.5 (C-11a), 97.3 (C-10a), 123, 129, 135, 136.5 (Ar-C), 146 (C-7a), 148 (C-10), 149 (C-3), 151 (C-11b), 152 (C-5), 155 (C-6a). MS *m/z* (%): 363 [M^+] (50.74). Anal. calcd. for $C_{17}H_{13}N_7O_3$: C 56.20, H 3.61, N 26.99. Found: C 56.34, H 3.63, N 26.83.

*9,9-Diethoxymethyl-5,10-dimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano [3,2-e][1,2,4]triazolo[4,3-c]pyrimidine **10***

Yield 37%, mp. 215 – 218°C. ^{1}H-NMR (DMSO-d_6): δ 1.05 (t, *J* = 9 Hz, 3H, OCH_2CH_3), 1.20 (t, *J* = 9 Hz, 3H, OCH_2CH_3), 1.96 (s, 3H, C^{10}-CH_3), 2.83 (s, 3H, C^5-CH_3), 4.12 (q, *J* = 9 Hz, 2H, CH_2CH_3), 4.4 (q, *J* = 9Hz, 2H, CH_2CH_3), 5.76 (s, 1H, pyran-H), 6.09 (s, 1H, $CH(OC_2H_5)_2$), 7.60 (d, *J* = 9 Hz, 2H, Ar-H), 8.13 (d, *J* = 9 Hz, 2H, Ar-H), 9.34 (s, 1H, C^3-H). MS *m/z* (%): 465 [M^+] (11.40), 420 [M^+-OC_2H_5] (2.91), 363 [(M^++H)-$CH(OC_2H_5)_2$] (8.8), 103 [$^+CH(OC_2H_5)_2$] (100). Anal. calcd. for $C_{22}H_{23}N_7O_5$: C 56.77, H 4.98, N 21.06. Found: C 56.90, H 4.90, N 21.01.

*5,10-Dimethyl-3-naphthalen-1-ylmethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine **11***

A mixture of compound **5** (3.53 g, 10 mmol) in phosphorus oxychloride (40 mL) and 1-naphthylacetic acid (1.86 g, 10 mmol) was heated under reflux temperature for 5 h. The reaction mixture was poured onto crushed ice, and then the obtained solid was filtered off, dried, and recrystallized from dioxane to give compound **11**. Yield 81%, mp. 222 – 223°C. IR ν 3220 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.9 (s, 3H, C^{10}-CH_3), 2.38 (s, 3H, C^5-CH_3), 4.0 (s, 2H, CH_2), 5.7 (s, 1H, pyran-H), 7.37 – 8.14 (m, 11H, Ar-H), 12.3 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 503 [M^+] (13.02). Anal. calcd. for $C_{28}H_{21}N_7O_3$: C 66.79, H 4.20, N 19.47. Found: C 66.84, H 4.17, N 19.45.

*Synthesis of compounds **12a – c***

General procedure: A solution of compound **5** (3.53 g, 10 mmol) in ethanol (50 mL), *p*-nitrobenzaldehyde (1.51 g, 10 mmol), *p*-methoxybenzaldehyde (0.12 mL, 10 mmol) in the presence of a few drops of piperidine or D-glucose (1.8 g, 10 mmol) in the presence a catalytic amount of acetic acid was heated at 80°C for 1 h. On cooling, a precipitate formed which was filtered off, dried, and recrystallized from appropriate solvent to give compounds **12a – c**, respectively.

*N-{3,7-Dimethyl-4-(p-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl}-N′-(p-nitrobenzylidene)hydrazine **12a***

Yield (90%, ethanol), mp. 333 – 335°C. IR ν 3163 (NH), 3113 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.96 (s, 3H, C^3-CH_3), 2.45 (s, 3H, C^7-CH_3), 6.05 (s, 1H, pyran-H), 7.41 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.94 (d, *J* = 9 Hz, 2H, Ar-H), 8.07 (d, *J* = 9 Hz, 2 H, Ar-H), 8.16 (s, 1H, N=CH), 8.30 (d, *J* = 8.4 Hz, 2H, Ar-H), 11.05 (s, 1H, NH, D_2O exchangeable), 12.24 (s, 1 H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 10.0 (C3-CH_3), 25.2 (C7-CH_3), 33.3 (C-4), 94.7 (C-4a), 98.0 (C-3a), 124-146 (Ar-C), 151 (C-3), 154.7 (C-9a), 159 (C-8a), 159.1 (N=CH), 164.4 (C-7), 165.9 (C-5). MS *m/z* (%): 486 [M^+] (16.36). Anal. calcd. for $C_{23}H_{18}N_8O_5$: C 56.79, H 3.73, N 23.03. Found: C 56.85, H 3.70, N 23.00.

*N-[3,7-Dimethyl-4-(p-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-N′-(p-methoxybenzylidene)hydrazine **12b***

Yield (92%, ethanol), mp. 283 – 285°C. ^{1}H-NMR (DMSO-d_6): δ 2.06 (s, 3H, C^3-CH_3), 2.41 (s, 3H, C^7-CH_3), 3.80 (s, 3H, OCH_3), 6.04 (s, 1H, pyran-H), 7.02 (d, *J* = 9 Hz, 2H, Ar-H), 7.42 (d, *J* = 9 Hz, 2H, Ar-H), 7.63 (d, *J* = 9 Hz, 2H, Ar-H), 8.02 (s, 1H, N=CH), 8.08 (d, *J* = 9 Hz, 2H, Ar-H), 10.53 (s, 1H, NH, D_2O exchangeable), 12.21 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6): δ 9.9 (C3-CH_3), 25.2 (C7-CH_3), 33.2 (C-4), 55 (OCH_3), 94.7 (C-4a), 98 (C-3a), 123 – 146 (Ar-C), 152 (C-3), 155 (C-9a), 159 (C-8a), 160 (N=CH), 164 (C-7), 165 (C-5). MS *m/z* (%): 471 [M^+] (17.77). Anal. calcd. for $C_{24}H_{21}N_7O_4$: C 61.14, H 4.49, N 20.80. Found: C 61.30, H 4.50, N 20.58.

*(2R,3R,4R,5S)-6-{[3,7-Dimethyl-4-(p-nitrophenyl)-2,4-dihydropyrazolo [4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-hydrazono}hexane-1,2,3,4,5-pentaol **12c***

Yield (95%, dioxane), mp. 133 – 135°C. IR ν 3411 – 3310 (broad NH, OH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.88 (s, 3H, C^3-CH_3), 2.33 (s, 3H, C^7-CH_3), 2.99 – 3.63 (some of the alditol protons congregated with water signals), 3.65 – 3.90 (m, 2H, CH_2OH), 4.20 – 4.70 (m, 2H, 2OH, D_2O exchangeable), 5.0 (m, 1H, OH, D_2O exchangeable), 5.30 (d, 1H, OH, D_2O exchangeable), 5.60 (s, 1H, pyran-H), 5.92 (d, 1H, OH, D_2O exchangeable), 7.33 – 7.59 (m, 3H, 2Ar-H + N=CH), 8.10 (d, *J* = 9 Hz, 2H, Ar-H), 10.40 (s, 1H, NH, D_2O exchangeable), 12.13 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 454 [M^+-(CH_2OH + CHOH)] (14.45). Anal. calcd. for $C_{22}H_{25}N_7O_8$: C 51.26, H 4.89, N 19.02. Found: C 51.31, H 4.90, N 18.96.

The attempted cyclization of compounds **12a-c** to their corresponding pyrazolo [4′,3′:5,6]pyrano[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **13a – c** by refluxing with bromine in glacial acetic acid did not succeed, however, unidentified products were isolated.

*5,10-Dimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo-[4′,3′:5,6]pyrano[3,2-e]tetrazolo[1,5-c]pyrimidine **14***

A cooled solution of compound **5** (3.53 g, 10 mmol) in diluted HCl (20 mL) was treated drop wise with a cooled solution of sodium nitrite (prepared from 1 g sodium nitrite dissolved in 15 mL water), then stirred at room temperature for 2 h. The separated solid was filtered off, dried, and recrystallized from ethanol to give compound **14**. Yield 87%, mp. 255 – 257°C. IR ν 3219 (NH) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.96 (s, 3H, C^{10}-CH_3), 2.99 (s, 3H, C^5-CH_3), 5.89 (s, 1 H, pyran-H), 7.63 (d, *J* = 9 Hz, 2 H, Ar-H), 8.15 (d, *J*

= 9 Hz, 2 H, Ar-H), 12.45 (s, 1 H, NH, D_2O exchangeable). MS *m/z* (%): 364 [M^+] (28). Anal. calcd. for $C_{16}H_{12}N_8O_3$: C 52.75, H 3.32, N 30.76. Found: C 52.64, H 3.21, N 30.98.

*9-(2-Methoxyethyl)-2,5,10-trimethyl-11-(p-nitrophenyl)-9,11-dihydropyrazolo[4′,3′:5,6]pyrano [2,3-e] [1,2,4]triazolo[1,5-c]pyrimidine **15***

A mixture of compound **6** (0.38 g, 1 mmol) and 50% oil-immersed sodium hydride (0.05 g, 0.2 mmol) in dry dimethylformamide (30 mL) was stirred at 70°C for 1 h, then cooled to room temperature. 2-Chloroethylmethyl ether (1 mmol) was added, and stirred at 90°C for 8 h. The mixture was evaporated under reduced pressure and chromatographed on a silica gel column with a chloroform : methanol mixture (9 : 1) as an eluent to give compound **15**. Yield 54%, oil. ^{1}H-NMR (DMSO-d_6): δ 2.08 (s, 3H, C^{10}-CH_3), 2.27 (s, 3H, C^5-CH_3), 2.73 (s, 3H, C^2-CH_3), 3.24 (s, 3H, OCH_3), 3.33 – 3.41 (m, 4H, CH_2CH_2), 5.40 (s, 1H, pyran-H), 7.04 (d, *J* = 9 Hz, 2H, Ar-H), 7.86 (d, *J* = 8.4 Hz, 2H, Ar-H). Anal. calcd. for $C_{21}H_{21}N_7O_4$: C 57.93, H 4.86, N 22.52. Found: C 58.01, H 4.81, N 22.49.

Antimicrobial activity of the newly synthesized compounds

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 microorganisms used were obtained from the Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt. Disc-diffusion sensitivity testing was done in the manner identical to that described by Bauer *et al.* [28]. Media for disc-sensitivity tests were nutrient agar and Müller – Hinton agar (MHA), purchased from Difco, USA. The non sterile powder of the tested compounds was dissolved in sterile DMSO to yield 2 μgmL^{-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 to Streptomycin as a reference drug.

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