Full Paper

Synthesis of Pyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine Derivatives for Antiviral Evaluation

Ahmed H. Shamroukh, Magdi E. A. Zaki, Eman M. H. Morsy, Faiza M. Abdel-Motti, and Farouk M. E. Abdel-Megeid

Photochemistry Department, National Research Centre, Dokki, Cairo, Egypt

6-Amino-3-methyl-4-(4-nitrophenyl)-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (**1**) was used as a precursor for preparation of some novel 3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine derivatives **3**–**6**, and some of their corresponding N^2- and C^5-*S*-acyclic nucleosides **7** and **8**. Furthermore, the preparation of 5-amino-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidin-5-yl]-1*H*-pyrazole derivatives **10**–**16** were described. Some of the prepared products were selected and tested for antiviral activity against Herpes Simplex Virus type-1 (HSV-1).

Keywords: Acyclic nucleosides / Antiviral activities / Pyranopyrazoles / Pyrazoles / Pyrazolopyranopyrimidines

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Introduction

The chemistry of pyrazole derivatives, especially 3-methyl-1-phenyl-2-pyrazolin-5-one, has received great interest, since this nucleus is the parent skeleton of pyrine drugs and other medicinal compounds [1–2]. Pyrano[2,3-*c*]pyrazole derivatives showed biological activities as bactericides [3] and virucides [4]. Moreover, a large number of fused pyrimidine derivatives exhibited antimycobacterial [5], antitumor [6], and antiviral activities [7]. In continuation of our previous work on pyrazoles [8–10], pyrano[2,3-*c*]pyrazoles [11], pyrimidines [12], and fused pyrimidines [9, 13–14], we intended to synthesize a pyrano[2,3-*c*]pyrazole ring system fused to a pyrimidine moiety to obtain new compounds which are expected to possess notable chemical and biological activities.

Results and discussion

Chemistry

This work deals with synthesis and study of the reactions of fused pyrano[2,3-*c*]pyrazoles from their corresponding β-enaminonitrile precursor: 6-amino-2,4-dihydro-3-methyl-4-(4-nitrophenyl)pyrano[2,3-*c*]pyrazole-5-carbonitrile **1** [11, 15] as key compound for this study and for further syntheses of other fused heterocyclic compounds. Thus, when compound **1** was stirred at room temperature with acetic anhydride it gave 6-acetamido-3-methyl-4-(4-nitrophenyl)-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile **2**. The structure of the latter compound was confirmed on the basis of its elemental analysis and spectral data (see Experimental and Scheme 1). The IR spectrum of compound **2** showed absorption bands assignable to the NH groups, CN-, and CO group. Its ^{1}H-NMR spectrum showed signals at 1.77 (s, 3H, C^3-CH_3), 2.24 (s, 3H, O=C-CH_3), 11.91, and 12.63 for 2 × NH (D_2O exchangeable) and MS gave the molecular ion peak at *m/z* (%): 339 [M^+] (42.72). While carrying out the same reaction under reflux, acetylation took place to give 2-acetyl-3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]-pyrano[2,3-*d*]pyrimidin-5(6*H*)-one **3**.

The structure of the aforementioned compound was confirmed by spectral data (see Experimental and Scheme 1). The ^{1}H-NMR spectrum showed a signal at 2.26 (s, 3H, C^3-CH_3). Actually, by surveying the alkylation and acylation reactions of pyrazole derivatives, we can conclude that N^2-acetylation which took place for compound **3**, is in accordance with results which judged that the ^{1}H-NMR chemical shifts of C^3-methyl group adjacent to N^2-

Correspondence: Ahmed H. Shamroukh, Photochemistry Department, National Research Centre, Dokki, Cairo 12622, Egypt.
E-mail: ahshamroukh@yahoo.com
Fax: +20 203 37-0931

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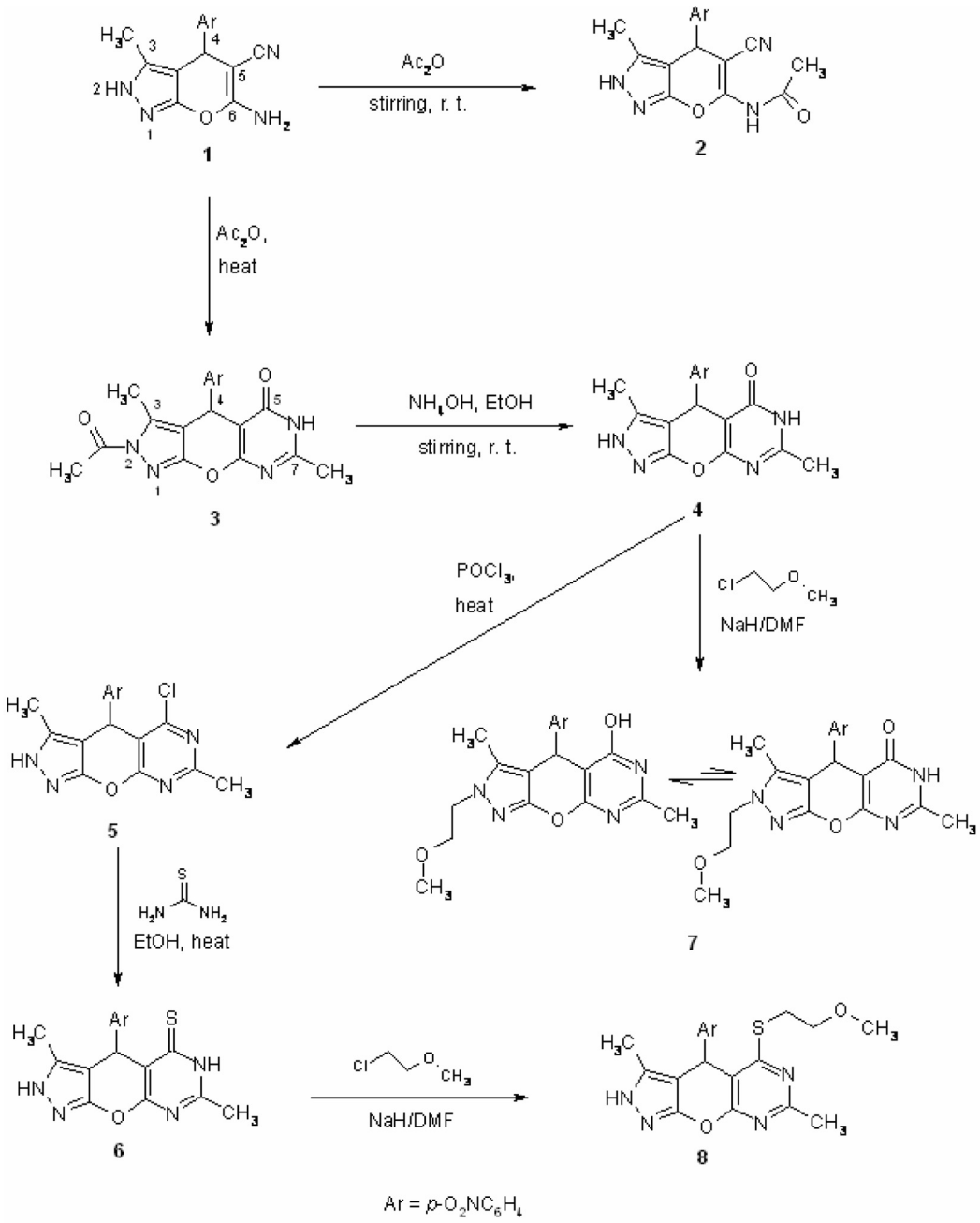

Scheme 1. Synthesis route of compounds **1 – 8**.

alkyl group are more deshielded and appear invariably at a lower field (~δ 2.25 – 2.30 ppm), when compared with that of the C^3-methyl protons of N^1-alkyl derivatives (~δ 1.9 – 2.0 ppm) [16, 17]. Furthermore, the ^{1}H-NMR data given by many authors for alkylation, acylation [11, 13, 16, 18 – 20] and nucleosidation reactions [21], the single crystal X-ray analysis proved no doubt that acetylation took place at the N^2-nitrogen of the pyrazole ring (Fig. 1, Table 1).

It was reported by Arakawa *et al*. [18] and Kashima [19] that formation of acyl pyrazoles is governed by the steric interaction between the ring substitution groups and the acyl moiety which isomerizes slowly from the N^2-position into the more thermodynamically stable N^1-acetyl product. However, when we attempted isomerization of product **3** into its corresponding N^1-acetyl product under very mild conditions, only deacetylation started to take place with formation of 3,7-dimethyl-4-(4-nitrophenyl)-2,4-

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Table 1. Crystal data, bond lengths, bond angles, and torsion angles of compound **3**.

Crystal system	**Monoclinic**	**Torsion angles** (°)	
Space group	P-1	C15–N4–N9–C22	−0.3 (7)
a (Å)	24.6358 (14)	N9–N4–C15–C24	−0.3 (6)
b (Å)	7.2915 (4)	N9–N4–C15–C29	−177.5 (11)
c (Å)	21.7147 (14))	C18–N4–N9–C22	176.2 (9)
α (°)	90.00°	N9–N4–C18–O23	−171.9 (11)
β (°)	93.600 (3)°	N9–N4–C18–C28	7.3 (7)
γ (°)	90.00°	C15–N4–C18–O23	3.9 (8)
V (Å^3)	3893.0 (4)Å^3	C18–N4–C15–C24	−176.3 (11)
Z	8	C15–N4–C18–C28	−176.9 (12)
D_x	1.452 Mg m^{-3}	C18–N4–C15–C29	6.4 (7)
R	0.043	C24–C15–N4–N9	−0.3 (6)
wR	0.076	C24–C15–N4–C18	−176.3 (11)
Bond length (Å)		C29–C15–N4–C18	6.4 (7)
N4–N9	1.386 (2)	O23–C18–N4–N9	−171.9 (11)
N4–C15	1.385 (2)	O23–C18–N4–C15	3.9 (8)
N4–C18	1.417 (2)	C28–C18–N4–N9	7.3 (7)
Bond angles (°)		C28–C18–N4–C15	−176.9 (12)
N9–N4–C15	112.42 (12)		
N9–N4–C18	118.6 (2)		
C15–N4–C18	128.9 (2)		
N4–N9–C22	100.69 (12)		
N4–C18–O23	119.6 (2)		
N4–C18–C28	115.7 (2)		
O23–C18–C28	124.7 (2)		

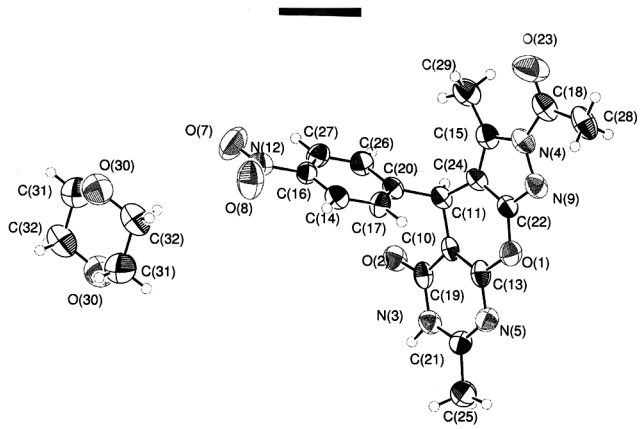

Figure 1. Single crystal X-ray structure of compound **3** and dioxane solvent.

dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidin-5(6*H*)-one **4** (see Experimental and Scheme 1). Compound **4** was converted into its corresponding 5-chloro derivative **5** (Scheme 1) by refluxing with phosphorus oxychloride. The mass spectra of compound **5** gave fragments showing the isotopic pattern due to the presence of chlorine atom (see Experimental). Compound **5** was converted into its corresponding 5-thiono derivative **6** (Scheme 1) following the procedure of Schiba *et al*. [22] and its ^{13}C-NMR spectrum showed a signal at d 183 ppm accountable for the C=S group (see Experimental).

Since the synthesis of acyclovir, as one of the potent antiherpetic drug by Schaffer *et al*. [23], many attempts have been directed by nucleoside chemists to prepare a lot of related compounds with various side chains and glycons [24, 25]. However in this connection, there is only one recent report in the literature [21] on the N^1-pyrazole acyclic nucleosides of pyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine derivatives which were tested for their anti-inflammatory activity. The N^2-acyclic nucleosides and *S*-acyclic nucleosides of pyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine derivatives are still not known (to the best of our knowledge) in the literature. Thus, in continuation of our previous work [9, 26] in preparing various cyclic and acyclic nucleosides of different heterocyclic compounds, in this report, we describe the synthesis of some N^2- and *S*-acyclic nucleosides related to pyrazolo[4′,3′:5,6]-pyrano[2,3-*d*]pyrimidine derivatives by treating the sodium salt of compounds **4** and **6** (generated *in situ*, see Experimental) with 2-chloroethyl methyl ether; they afforded the corresponding N^2- and C^5-*S*-acyclic nucleosides: 3,7-dimethyl-2-(2-methoxyethyl)-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidin-5-ol **7** and 5-(2-methoxy-ethylsulfanyl)-3,7-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyrazolo[4′,3′:5,6]pyrano[3,2-*d*]pyrimidine **8**, respectively (Scheme 1). The structural assignments and sites of nucleosidation of compounds **7** and **8** were assigned on the basis of their elemental and spectral data (see Experimental and Scheme 1). The ^{1}H-NMR spectrum

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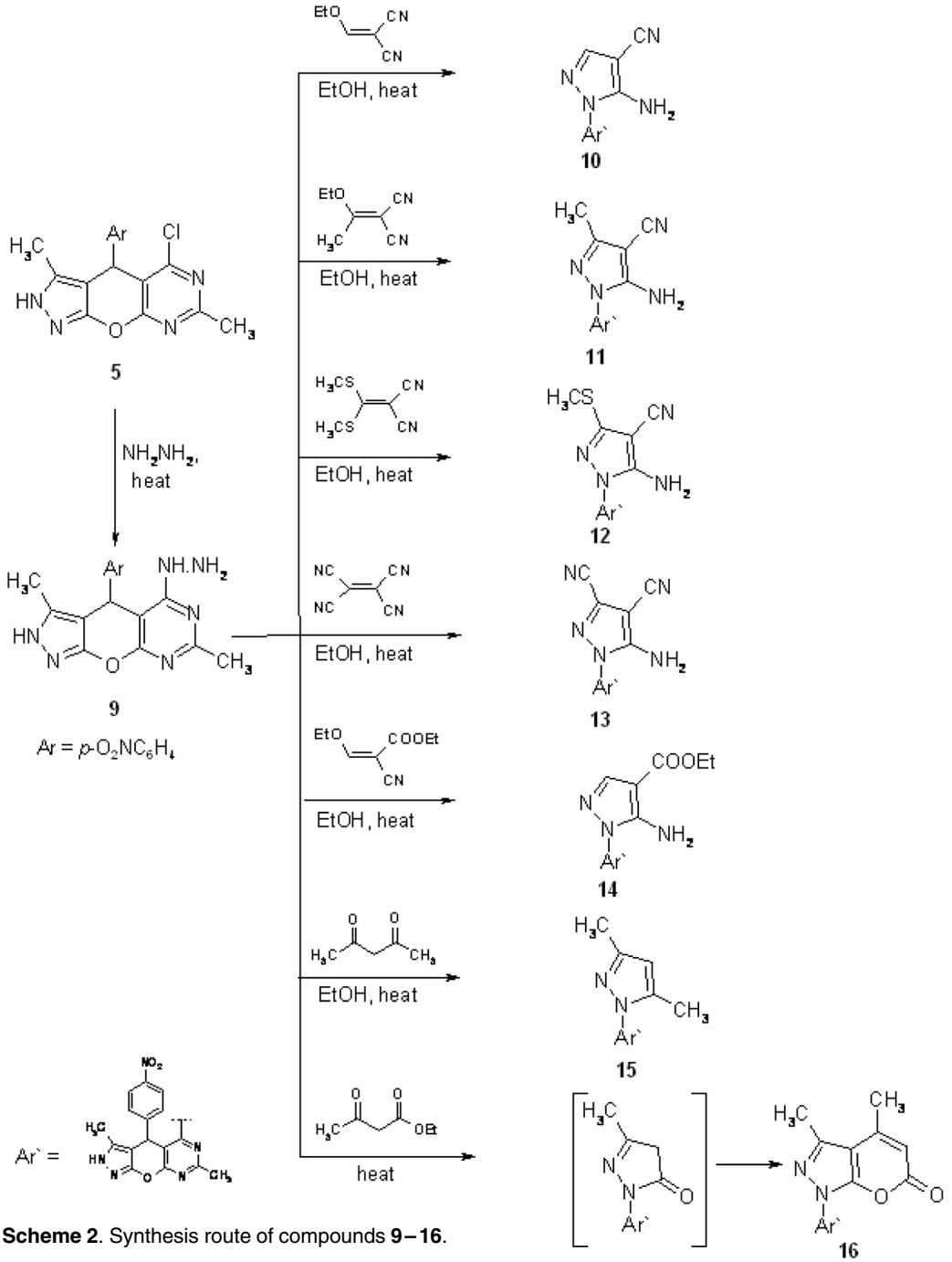

Scheme 2. Synthesis route of compounds **9 – 16**.

of compound **7** revealed the absence of signals at δ 12.18, 12.40 ppm of the NH of the pyrazole and the pyrimidine ring, however a signal at δ 6.79 ppm assignable to the OH of the pyrimidine moiety due to the tautomerization. The assignment of the N^2- nucleosidation was judged by the appearance of the C^3-CH_3 protons at δ 2.25 ppm, and this is in accordance with reported results for N^2-alkylation of related compounds [16]. In compound **8**, the presence of NH at δ 11.30 ppm (exchangeable with D_2O) in ^{1}H-NMR spectrum and the absence of the C=S group in its ^{13}C-NMR spectrum proved that nucleosidation took place at the C^5-*S*-pyrimidine. Deprotection of nucleosides **7** and **8** (using ammonium hydroxide solution/ethanol) afforded again compounds **4** and **6**, respectively.

When compound **5** was heated with hydrazine hydrate, it gave a compound assigned to the structure of

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3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo-[4′,3′:5,6]pyrano[2,3-*d*]pyrimidin-5-ylhydrazine **9** (Experimental, Scheme 2). When the compound **9** was refluxed with ethoxymethylenemalononitrile, methylethoxymethylenemalononitril, bis-(methylthio)methylenemalononitrile, tetracyanoethylene or ethyl(ethoxy-methylene)cyano-acetate, it afforded the corresponding substituted pyrazole derivatives **10**–**14**, respectively (Scheme 2). The structures of the latter compounds were confirmed on the basis of their elemental analysis and spectral data (Experimental). The IR spectra of compounds **10**–**13** showed absorption bands characteristic for NH_2 and CN groups, while compound **14** revealed absorption bands characteristic for NH_2 and C=O. Also, ^{1}H-NMR spectra showed signals at δ = 6.79, 6.75, 6.85, 6.55, and 6.53 ppm due to NH_2 (exchangeable with D_2O) for compounds **10**–**14**, respectively. The MS gave the molecular ion peaks at *m/z* (%) = 429 (23.49), 443 (20.85), 475 (3.25), 454 (18.30), and 476 (41.88) for compounds **10**–**14**, respectively.

Similarly, when a mixture of compound **9** and acetylacetone in ethanol was refluxed, it gave 5-(3,5-dimethylpyrazol-1-yl)-3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine **15** (Experimental and Scheme 2). The ^{1}H-NMR spectrum of the latter compound showed signals at δ = 1.75 ppm and 2.25 ppm for ($2 \times CH_3$). The MS, gave the molecular ion peak as a base peak at *m/z* (%) = 417 (100).

On the other hand, when a mixture of compound **9** and excess of ethyl acetoacetate was refluxed, it lead to an unexpected product assigned to the structure of 1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]-pyrano[2,3-*d*]pyrimidin-5-yl]-3,4-dimethyl-3a,7a-dihydro-1*H*-pyrano[2,3-*c*]pyrazol-6-one **16** (Experimental, Scheme 2). The formation of compound **16** might have taken place via the formation of the pyrazolone intermediate which reacted further with another mole of ethyl acetoacetate. The ^{1}H-NMR spectrum of compound **16** showed signals at 2.35 ppm, 2.51 ppm for ($2 \times CH_3$), and 5.69 ppm for (pyranyl-H), and its IR spectrum revealed the presence of a C=O group. The MS gave the molecular ion peak as a base peak at *m/z* (%) = 485 (100).

Antiviral Screening

The plaque infectivity assay was carried out to test compounds **3, 4**, **6, 8, 10, 11, 12, 13**, and **16** for antiviral activity. The test was performed to include the three possibilities for antiviral activity – viricidal effect, virus adsorption, and effect on virus replication for HSV-1. The results are summarized in Fig. 2. Compound **12** revealed a toxic effect on viral growth. On the other hand, compounds **3, 4**, **6**, and **8** revealed the highest anti-HSV-1 activity in comparison with acyclovir as control. At both concentration, all tested compounds revealed no higher antiviral activity than acyclovir. In general, compound **4** showed the highest effect on HSV-1 than the other tested compounds, where its antiviral activity increased from 63% at concentration of 20 μg/10^5 cells to 95% at concentration of 40 μg/10^5 cells.

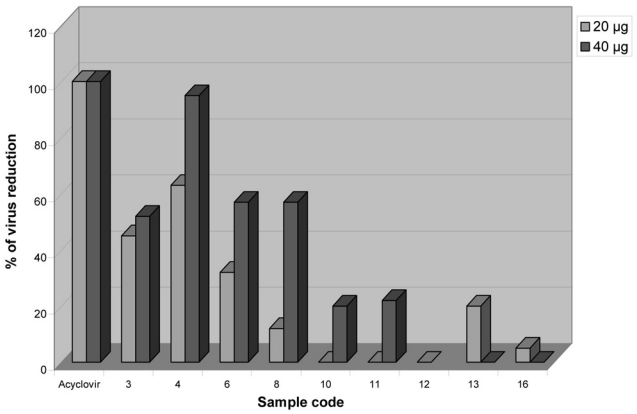

Figure 2. Effect of novel derivatives on HSV-1.

Conclusion

Some novel pyrazolo[4′,3′:5,6]pyrano[2,3-*d*]pyrimidine derivatives **3**–**6**, and some of their corresponding N^2- and C^5-*S*-acyclic nucleosides **7** and **8** were prepared. Furthermore, some pyrazole derivatives **10**–**16** were described. Structure-activity correlation of the obtained results revealed that the pyrazolopyranopyrimidine ring, in compounds **3, 4**, **6**, and **8** showed promising antiviral activity. When the acetyl group of compound **3** was removed, it was noticed that the new pyrazolopyranopyrimidine derivative **4** was found to be more active as antiviral agent for HSV-1 (due to the two free NH groups). While replacement of oxygen (in compound **4**) with sulfur (in compound **6**), decreased the activity. Addition of another ring e.g., a pyrazole ring in compounds **10, 11, 12, 13**, and **16** decreased the activity% of HSV-1 reduction.

We are thankful to Dr. M. A. Ali, Virology Laboratory, National Research Centre for antiviral evaluation.

Experimental

Chemistry

All melting points are measured using Electro-thermal IA 9100 apparatus, (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin Elmer, USA), National Research Centre, Cairo,

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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 (d 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 chromatography was performed on Silica gel 60 (particle size 0.06 – 0.20 mm; Merck, Darmstadt, Germany).

Compound **1** was prepared according to a reported method [11].

*Synthesis of compounds **2, 3, 4, 5**, and **6***

*6-Acetamido-3-methyl-4-(4-nitrophenyl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile **2***

A solution of compound **1** (2.97 g, 1 mmol) in acetic anhydride (10 mL) was stirred at room temperature for 10 h. The formed precipitate was filtered off, dried, and recrystallized from ethanol to give compound **2**. Yield 89%, m.p. 240 – 243°C. IR (KBr, cm^{-1}) 3315 (NH), 3201 (NH), 2200 (CN) and 1735 (CO). ^{1}H-NMR (DMSO-d$_6$) δ 1.77 (s, 3H, C^3-CH$_3$), 2.24 (s, 3H, CO-CH$_3$), 4.98 (s, 1H, pyran), 7.58 (d, *J* = 12 Hz, 2H, Ar-H), 8.23 (d, *J* = 13.08 Hz, 2H, Ar-H), 11.91 (s, 1H, NH, D$_2$O exchangeable), and 12.63 (s, 1H, NH, D$_2$O exchangeable). MS *m/z* (%): 339 [M$^+$] (42.72). Anal. calcd. for $C_{16}H_{13}N_5O_4$: C 56.64, H 3.86, N 20.64. Found: C 56.53, H 3.90, N 20.71.

*2-Acetyl-3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(6H)-one **3***

A solution of compound **1** (2.97 g, 1 mmol) in acetic anhydride (10 mL) was refluxed for 3 h. The reaction mixture was evaporated till dryness and the remaining solid was recrystallized from dioxane to give compound **3**. Yield 50%, m.p. 356 – 358°C. IR (KBr, cm^{-1}) 3440 (NH), 1735 (CO) and 1650 (CO). ^{1}H-NMR (DMSO-d$_6$) δ 2.26 (s, 3H, C^3-CH$_3$), 2.31 (s, 3H, C^7-CH$_3$), 2.56 (s, 3H, COCH$_3$), 5.28 (s, 1H, pyran), 7.56 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.14 (d, *J* = 8.7 Hz, 2H, Ar-H) and 12.65 (s, 1H, NH, D$_2$O exchangeable). ^{13}C-NMR (DMSO-d$_6$) δ 13.0 (C3-CH$_3$), 21.0 (C7-CH$_3$), 23.1 (CH$_3$-acetyl), 33.3 (C-4), 99 (C-4a), 106.6 (C-3a), 123, 129, 140, 146 (Ar-C), 150 (C-3), 155 (C-9a), 159.6 (C-8a), 161 (C-7), 162.4 (C-5), 171 (CO). MS *m/z* (%): 381 [M$^+$] (25.41). Anal. calcd. for $C_{18}H_{15}N_5O_5$: C 56.69, H 3.96, N 18.36. Found: C 56.73, H 4.01, N 18.27.

*3,7-Dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo-[4',3':5,6]pyrano[2,3-d]pyrimidin-5(6H)-one **4***

A mixture of compound **3** (3.81 g, 1 mmol) and ammonium hydroxide solution (1 mL, 25%) in ethanol (30 mL) was stirred at room temperature for 3 h. The formed precipitate was filtered off, washed with water several times, and recrystallized from dioxane to give compound **4.** Yield 89%, m.p. 390 – 392°C IR (KBr, cm^{-1}) 3164 (NH), 3200 (NH) and 1653 (CO). ^{1}H-NMR (DMSO-d$_6$) δ 1.90 (s, 3H, C^3-CH$_3$), 2.28 (s, 3H, C^7-CH$_3$), 5.14 (s, 1H, pyran), 7.46 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.7 Hz, 2H, Ar-H), 12.18 (s, 1H, NH, D$_2$O exchangeable) and 12.40 (s, 1H, NH, D$_2$O exchangeable). ^{13}C-NMR (DMSO-d$_6$) δ 9.8 (C3-CH$_3$), 20.9 (C7-CH$_3$), 34 (C-4), 98 (C-4a), 99.5 (C-3a), 123, 129, 136, 145 (Ar-C), 152 (C-3), 155 (C-9a), 158 (C-8a), 162 (C-7), 162,8 (C5). MS *m/z* (%): 339 [M$^+$] (18.10). Anal. calcd. for $C_{16}H_{13}N_5O_4$: C 56.64, H 3.86, N 20.64. Found: C 56.51, H 3.90, N 20.73.

*5-Chloro-3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine **5***

A mixture of compound **4** (0.339 g, 1 mmol) in phosphorus oxychloride (10 mL) was heated for 2 h. The solution was cooled and poured into ice-water, and the formed precipitate was filtered off, dried, and recrystallized from dioxane to give compound **5**. Yield 85%, m.p. 340 – 342°C. ^{1}H-NMR (DMSO-d$_6$) δ 2.00 (s, 3H, C^3-CH$_3$), 2.49 (s, 3H, C^7-CH$_3$), 5.59 (s, 1H, pyran), 7.52 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.13 (d, *J* = 8.7 Hz, 2H, Ar-H) and 12.29 (s, 1H, NH, D$_2$O exchangeable). MS *m/z* (%): 359 [M$^+$] Cl^{37}, (3.66), 357 [M$^+$] Cl^{35}, (9.81). Anal. calcd. for $C_{16}H_{12}ClN_5O_3$: C 53.72, H 3.38, Cl 9.91, N 19.58. Found: C 53.85, H 3.31, Cl 10.02, N 19.41.

*3,7-Dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo-[4',3':5,6]pyrano[2,3-d]pyrimidine-5(6H)-thione **6***

A solution of compound **5** (3.57 g, 1 mmol) and thiourea (1.52 g, 2 mmol) in ethanol (20 mL) was heated at reflux for 4 h. The formed precipitate was filtered off, dried, and recrystallized from dimethylformamide to give compound **6**. Yield 77%, m.p. 380 – 382°C. IR (KBr, cm^{-1}) 3250 (NH), and 3225 (NH). ^{1}H-NMR (DMSO-d$_6$) δ 2.01 (s, 3H, C^3-CH$_3$), 2.43 (s, 3H, C^7-CH$_3$), 5.47 (s, 1H, pyran), 7.47 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.07 (d, *J* = 8.7 Hz, 2H, Ar-H), 12.16 (s, 1H, NH, D$_2$O exchangeable) and 13.97 (s, 1H, NH, D$_2$O exchangeable). ^{13}C-NMR (DMSO-d$_6$) δ 9.8 (C3-CH$_3$), 20 (C7-CH$_3$), 37 (C-4), 98 (C-4a), 112 (C-3a), 123, 129, 136, 145 (Ar-C), 151 (C-3), 154 (C-9a), 158 (C-8a), 161 (C-7), 183 (C-5). MS *m/z* (%): 355 [M$^+$] (78.38). Anal. calcd. for $C_{16}H_{13}N_5O_3S$: C 54.08, H 3.69, N 19.71, S 9.02. Found: C 54.00, H 3.75, N 19.63, S 9.12.

*Synthesis of compounds **7** and **8**; general procedure*

A mixture of compound **4** (0.34 g, 0.1 mmol) or **6** (0.36 g, 0.1 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. The solution was cooled, and then 2-chloroethylmethyl ether (0.1 mmol) was added and stirred at 90°C for 8 h. The reaction mixtures were evaporated under reduced pressure and chromatographed on silica gel column using chloroform/methanol mixture (9 : 1) as an eluent to give compounds **7** or **8**, respectively.

*3,7-Dimethyl-2-(2-methoxyethyl)-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5-ol **7***

Yield 49%. Oil. IR (KBr, cm^{-1}) 3420 – 3300 (broad, OH). ^{1}H-NMR (DMSO-d$_6$) δ 2.25 (s, 3H, C^3-CH$_3$), 2.29 (s, 3H, C^7-CH$_3$), 3.0 (s, 3H, OCH$_3$), 3.52 – 3.60 (m, 4H, CH$_2$CH$_2$), 5.49 (s, 1H, pyran-H), 6.79 (brs, 1H, OH, D$_2$O exchangeable), 7.12 (d, *J* = 9.0 Hz, 2H, Ar-H), and 7.65 (d, *J* = 8.4 Hz, 2H, Ar-H). Anal. calcd. for $C_{19}H_{19}N_5O_5$: C 57.43, H 4.82, N 17.62. Found: C 57.51, H 4.77, N 17.59.

*5-(2-Methoxyethylsulfanyl)-3,7-dimethyl-4-(4-nitrophenyl)–2,4-dihydropyrazolo[4',3':5,6]pyrano[3,2-d]-pyrimidine **8***

Yield 72%, m.p. 237 – 240°C. ^{1}H-NMR (DMSO-d$_6$) δ 1.80 (s, 3H, C^3-CH$_3$), 2.25 (s, 3H, C^7-CH$_3$), 3.00 (s, 3H, OCH$_3$), 3.21-3.29 (m, 4H, CH$_2$CH$_2$), 4.85 (s, 1H, pyran-H), 7.05 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.80 (d, *J* = 8.4 Hz, 2H, Ar-H) and 11.30 (s, 1H, NH, D$_2$O exchangeable). ^{13}C-NMR (DMSO-d$_6$) δ 9.9 (C3-CH$_3$), 25 (C7-CH$_3$), 29 (C-4), 35.7 (S-CH$_2$), 57.8 (O-CH$_3$), 70 (CH$_2$-O), 97 (C-4a), 108 (C-3a), 123, 129, 136, 146 (Ar-C), 150 (C-3), 154 (C-9a), 162 (C-8a), 165 (C-7), 170 (C-5).

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Anal. calcd. for $C_{19}H_{19}N_5O_4S$: C 55.20, H 4.63, N 16.94, S 7.76. Found: C 55.25, H 4.68, N 17.00, S 7.60.

*Attempted deprotection of compounds **7** and **8***

To a solution of compounds **7** (0.19 g, 0.5 mmol) or **8** (0.20 g. 0.5 mmol) in ethanol (25 mL), ammonium hydroxide solution (20 mL, 25%) was added with stirring at room temperature for 1 h. The isolated solids were filtered off, and dried to give compounds identical in all aspects with compounds **4** and **6**, respectively.

*3,7-Dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo-[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-ylhydrazine **9***

To a solution of compound **5** (3.59 g, 1 mmol) in dry dioxane (30 mL), hydrazine hydrate (3 mL, 99%) was added and the reaction mixture was heated on a water bath for 1 h. After cooling the precipitated material was filtered off, washed several times with water, dried and recrystallized from ethanol to give compound **9**. Yield 99%, m.p. 273–274°C. IR (KBr, cm^{-1}) 3420–3238 (NH_2, NH). ^{1}H-NMR (DMSO-d_6) δ 1.97 (s, 3H, C^3-CH_3), 2.37 (s, 3H, C^7-CH_3), 4.34 (s, 2H, NH_2, D_2O exchangeable), 5.37 (s, 1H, pyran), 7.99 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.81(s, 1H, NH, D_2O exchangeable), 8.13 (d, *J* = 9.0 Hz, 2H, Ar-H), and 12.06 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 353 [M^+] (20.61). Anal. calcd. for $C_{16}H_{15}N_7O_3$: C 54.39, H 4.28, N 27.75. Found: C 54.22, H 4.32, N 27.88.

*5-Amino-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydro-pyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-3,4-disubstituted-1H-pyrazoles **10–14**; general procedure*

To a solution of compound **7** (3.53 g, 1 mmol) in ethanol (30 mL), ethoxymethylenemalononitrile, methylethoxymethylenemalononitril, bis(methylthio)methylene-malononitrile, tetracyanoethylene, or ethyl(ethoxymethylene)cyanoacetate (1 mmol) was added, respectively. The reaction mixture was heated for 2–4 h. Then the formed precipitate was filtered off and recrystallized from appropriate solvent to give compounds **10**–**14**.

*5-Amino-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-1H-pyrazole-4-carbonitrile **10***

Yield after 2 h: (96%, dioxane); m.p. 322–325°C. IR (KBr, cm^{-1}) 3430, 3300 (NH_2), 3220 (NH), 2222 (CN). ^{1}H-NMR (DMSO-d_6) δ 1.89 (s, 3H, $C^{3'}$-CH_3), 2.59 (s, 3H, $C^{7'}$-CH_3), 5.94 (s, 1H, pyran), 6.79 (s, 2H, NH_2, D_2O exchangeable), 7.15 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.82 (s, 1H, pyrazole-H), 8.01 (d, *J* = 8.7 Hz, 2H, Ar-H), and 12.30 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 429 [M^+] (23.49). Anal. calcd. for $C_{20}H_{15}N_9O_3$: C 55.94, H 3.52, N 29.36. Found: C 56.06, H 3.44, N, 29.32.

*5-Amino-3-methyl-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-1H-pyrazole-4-carbonitrile **11***

Yield after 4 h: (93%, dioxane); m.p. 342–344°C. IR (KBr, cm^{-1}) 3450, 3350 (NH_2), 3235 (NH), and 2220 (CN). ^{1}H-NMR (DMSO-d_6) δ 1.90 (s, 3H, $C^{3'}$-CH_3), 1.99 (s, 3H, C^3-CH_3), 2.58 (s, 3H, $C^{7'}$-CH_3), 6.04 (s, 1H, pyran), 6.75 (s, 2H, NH_2, D_2O exchangeable), 7.15 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.88 (d, *J* = 8.7 Hz, 2H, Ar-H) and 12.42 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 443 [M^+] (20.85). Anal. calcd. for $C_{21}H_{17}N_9O_3$: C 56.88, H 3.86, N 28.43. Found: C 56.80, H 3.92, N 28.45.

*5-Amino-3-methylthio-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-1H-pyrazole-4-carbonitrile **12***

Yield after 3 h: (84%, dioxane); m.p. 209–211°C. IR (KBr, cm^{-1}) 3313, 3299 (NH_2), 3200 (NH) and 2222 (CN) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.90 (s, 3H, $C^{3'}$-CH_3), 2.60 (s, 3H, $C^{7'}$-CH_3), 3.76 (s, 3H, SCH_3), 5.99 (s, 1H, pyran), 6.85 (s, 2H, NH_2, D_2O exchangeable), 7.16 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.95 (d, *J* = 8.7 Hz, 2H, Ar-H), and 12.35 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 475 [M^+] (3.25). Anal. calcd. for $C_{21}H_{17}N_9O_3S$: C 53.05, H 3.60, N 26.51, S 6.74. Found: C 53.16, H 3.64, N 26.38, S 6.72.

*5-Amino-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-1H-pyrazole-3,4-dicarbonitrile **13***

Yield after 4 h: (93%, ethyl acetate); m.p. 277–279°C. IR (KBr, cm^{-1}) 3436, 3350 (NH_2), 3161 (NH), and 2229 (CN). ^{1}H-NMR (DMSO-d_6) δ 1.89 (s, 3H, $C^{3'}$-CH_3), 2.59 (s, 3H, $C^{7'}$-CH_3), 6.12 (s, 1H, pyran), 6.55 (s, 2H, NH_2, D_2O exchangeable), 7.15 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.01 (d, *J* = 9.0 Hz, 2H, Ar-H) and 12.39 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 454 [M^+] (18.30). Anal. calcd. for $C_{21}H_{14}N_{10}O_3$: C 55.51, H 3.11, N 30.82. Found: C 55.64, H 3.04, N 30.76.

*Ethyl-5-amino-1-[3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-1H-pyrazole-4-carboxylate **14***

Yield after 4 h: (95%, dioxane); m.p. 272–274°C. IR (KBr, cm^{-1}) 3436, 3350 (NH_2), 3150 (NH) and 1683 (CO). ^{1}H-NMR (DMSO-d_6) δ 1.23 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 1.90 (s, 3H, $C^{3'}$-CH_3), 2.60 (s, 3H, $C^{7'}$-CH_3), 4.15 (q, *J* = 6.9 Hz, 2H, CH_2CH_3), 6.12 (s, 1H, pyran), 6.53 (s, 2H, NH_2, D_2O exchangeable), 7.17 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.75 (s, 1H, pyrazole-H), 7.96 (d, *J* = 8.7 Hz, 2H, Ar-H), and 12.32 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 476 [M^+] (41.88). Anal. calcd. for $C_{22}H_{20}N_8O_5$: C 55.46, H 4.23, N 23.52. Found: C 55.52, H 4.20, N 23.54.

*5-(3,5-Dimethylpyrazol-1-yl)-3,7-dimethyl-4-(4-nitrophenyl)-2,4-dihydro-pyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidine **15***

A mixture of compound **9** (3.53 g, 1 mmol), acetylacetone (1 mmol) in anhydrous ethanol (30 mL) was refluxed for 5 h. The reaction mixture was allowed to cool and the formed precipitate was filtered off, dried, and recrystallized from ethanol to give compound **15**. Yield 65%, m.p. 337–340°C. ^{1}H-NMR (DMSO-d_6) δ 1.75 (s, 3H, $C^{3'}$-CH_3), 1.86 (s, 3H, C^3-CH_3), 2.25 (s, 3H, $C^{5'}$-CH_3), 2.58 (s, 3H, C^7-CH_3), 5.80 (s, 1H, pyran-H), 5.97 (s, 1H, pyrazole-H) 7.06 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.95 (d, *J* = 9.0 Hz, 2H, Ar-H), and 12.27 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 417 [M^+] (100). Anal. calcd. for $C_{21}H_{19}N_7O_3$: C 60.43, H 4.59, N 23.49. Found: C 60.33, H 4.62, N 23.56.

*1-[3,7-Dimethyl-4-(4-nitrophenyl)-2,4-dihydropyrazolo-[4′,3′:5,6]pyrano[2,3-d]pyrimidin-5-yl]-3,4-dimethyl-3a,7a-dihydro-1H-pyrano[2,3-c]pyrazol-6-one **16***

A solution of compound **9** (3.53 g, 1 mmol) in ethyl acetoacetate (30 mL) was refluxed for 5 h. The mixture was evaporated under reduced pressure and the obtained oil washed several time with diethyl ether and recrystallized from ethanol to give compound

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16. Yield 66%, m.p. 307 – 310°C. ^{1}H-NMR (DMSO-d_6) δ 1.82 (s, 3H, $C^{3'}$-CH_3), 2.35 (s, 3H, C^3-CH_3), 2.51 (s, 3H, C^4-CH_3), 2.63 (s, 3H, $C^{7'}$-CH_3), 5.69 (s, 1H, pyran-H), 5.71 (s, 1H, pyran-H) 7.15 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.89 (d, *J* = 9.0 Hz, 2H, Ar-H) and 12.35 (s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 485 [M^+] (100). Anal. calcd. for $C_{24}H_{19}N_7O_5$: C 59.38, H 3.94, N 20.20. Found: C 59.51, H 4.01, N 20.00.

Antiviral screening

Preparation of synthetic compounds for bioassay

Tested compounds were dissolved as 10 mg each in 1 mL of 10% DMSO in water. The final concentration was 10 µg/µL (stock solution). The dissolved stock solutions were decontaminated by addition of 10 mL antibiotic-antimycotic mixture (10 000 U penicillin G sodium, 10 000 µg streptomycin sulfates and 250 µg amphotericin B, PAA Laboratories GmbH, Austria).

Cell culture

African green monkey kidney-derived cells (Vero) were used. Cells were propagated in Dulbeccos' Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic mixture. The pH was adjusted at 7.2 – 7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2 µm pore size nitrocellulose membrane. Viruses: Herpes Simplex Virus type-1 was obtained from Environmental Virology Lab., Department of Water Pollution Research, National Research Centre, Cairo, Egypt

Cytotoxicity assay

Cytotoxicity was assayed for both dimethylsulfoxide (DMSO) and the tested compounds. Serial dilutions were prepared and inoculated on Vero cells grown in 96-well tissue culture plates. The maximum tolerated concentration (MTC) for each compound was determined by both cell morphology and cell viability by staining with trypan blue dye.

Plaque reduction infectivity assay

A 6-well plate was cultivated with cell culture (10^5 cell/mL) and incubated for 2 days at 37°C. HSV-1 was diluted to give 10^4 PFU/mL and mixed with the tested compound at 10 and 20 µg concentrations and incubated overnight at 4°C. Growth medium was removed from the multiwell plate and virus-compound mixture was inoculated (100 µL/well). After 1 h contact time, the inoculum's was aspirated and 3 mL of MEM with 1% agarose was overlaid the cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formalin solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without chemical compounds. A standard acyclovir (Sigma-Aldrich) was used at the same concentrations as the tested compounds. Virus plaques were counted and the percentage of reduction was calculated [27].

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