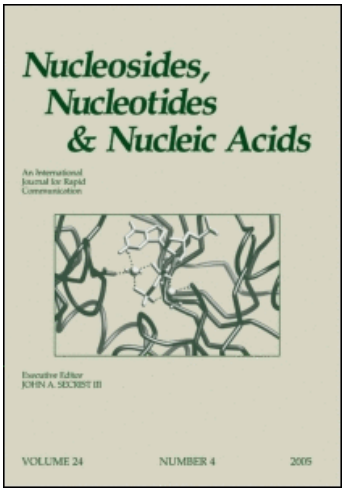

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Synthesis and Antiviral Activities of New Pyrazolo[4,3-*c*]quinolin-3-ones and Their Ribonucleoside Derivatives

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Synthesis and Antiviral Activities of New Pyrazolo[4,3-*c*]quinolin-3-ones and Their Ribonucleoside Derivatives

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ABSTRACT

Several new pyrazolo[4,3-*c*]quinolin-3-one ribonucleosides (**5a**–**g**) and their corresponding heterocycle moieties (**3a**–**g**) were synthesized and evaluated against vaccinia virus (VV) and herpes simplex virus type 1 (HSV-1). The derivatives **3c** and **3d** showed modest inhibitory activity against vaccinia virus reaching 70% at a concentration of 100 μM. All heterocyclic compounds (**3a**–**f**) showed a modest inhibition against HSV-1, reaching the maximal inhibitory effect around 20–30%.

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The antiviral effects of most of the pyrazolo[4,3-*c*]quinolin-3-one ribonucleosides (**5a**–**f**) on VV and HSV were not impressive.

Key Words: Antiviral activities; Pyrazolo[4,3-*c*]quinolinone; Ribonucleoside derivatives; Vaccinia virus; HSV-1.

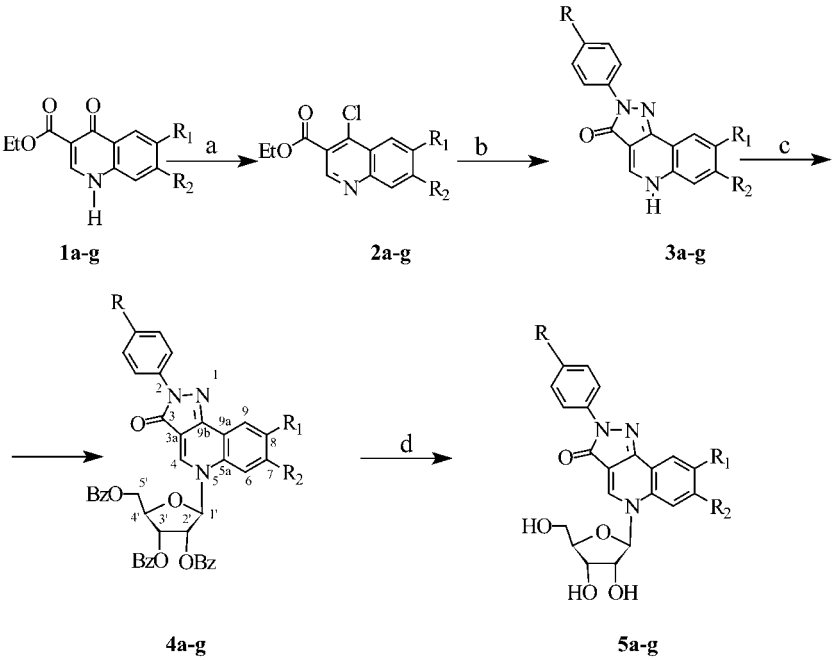
INTRODUCTION

Pyrazole derivatives have been found to exhibit biological activities such as antibacterial, anti-inflammatory, hypotensive and antitumor properties (Paul, S.; Gupta, M.; Gupta, R.; Loupy, A. Microwave assisted solvent-free synthesis of pyrazolo[3,4-*b*]quinolines and pyrazolo[3,4-*c*]pyrazoles using p-TsOH. Tetrahedron Lett. **2001**, *42*, 3827–3829). Condensed pyrazoles with other heterocyclic systems are known to possess various important biological activities e.g. some 2-phenylpyrazolo[4,3-*c*]quinolin-3-ones are ligands for benzodiazepine receptors (Fryer, R.I.; Zhang, P.; Rios, R.; Gu, Z.Q.; Basile, A.S.; Skolnick, P. Structure-activity relationship studies at the benzodiazepine receptor (BZR). A comparison of the substituent effects of pyrazoloquinolinone analogs. J. Med. Chem. **1993**, *36* (11), 1669–1673). Pyrazolo[3,4-*b*]quinoline derivatives also possess antiviral (Smirnoff, P.; Crenshaw, R.R. Stimulation of interferon-production in mice and in mouse spleen leukocytes by analogs by BL-20803. Antimicrob. Agents Chemother. **1977**, *11* (3), 571–573) and antimalarial (Stein, R.G.; Beil, J.H.; Singh, T. Antimalarials 4-substituted 1H-pyrazolo-3,4-betaquinolines. J. Med. Chem. **1970**, *13* (1), 153) activities and pyrazolo[3,4-*c*]pyrazole derivatives are useful antitumor agents. (Taylor, E.C.; Patel, H.; Kumar, H. Synthesis of pyrazolo[3,4-d]pyrimidine analogs of the potent antitumor agent N-(4[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl-ethyl]benzoyl)-L-glutamic acid-(LY231514). Tetrahedron **1992**, *48* (37), 8089–8100). As part of an ongoing program for the synthesis of potential antiviral agents based on the fused pyrazoloquinoline system, we prepared and investigated the antiviral and cytotoxic activities of several new pyrazolo[4,3-*c*]quinolin-3-one derivatives (**3a**–**g**), as well as their respective ribonucleosides (**5a**–**g**).

RESULTS AND DISCUSSION

The quinolone derivatives **1a**–**g** were prepared according to a procedure described in the literature,[1] which includes treatment of the appropriate aniline with diethyl ethoxymethylenemalonate to obtain the enamine derivatives that were then thermally cyclized in Dowtherm A.[2] Refluxing these quinolones in thionyl chloride afforded the corresponding chloro-derivatives **2a**–**g**. By using a slightly modified procedure[3,4] described in the literature, which involves the reaction of **2a**–**g** with the desired phenylhydrazine in toluene followed by refluxing in acetic acid, the corresponding 2-phenylpyrazolo[4,3-*c*]quinolin-3-ones **3a**–**g** were obtained in 78%, 85%, 70%, 80%, 74%, 72%, 84% yields, respectively (Scheme 1).

None of our attempts to silylate **3a**–**g** in a refluxing mixture of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMSCl)[5,6] were successful. Heating **3a**–**g** with bis(trimethylsilyl)trifluoroacetamide (BSTFA)[7–9] containing 1% of TMSCl at



1a-g **2a-g** **3a-g**

4a-g **5a-g**

a- R_1= Me, R_2= H, R= H
b- R_1= Me, R_2= H, R= OMe
c- R_1= Me, R_2= H, R= F
d- R_1= Me, R_2= H, R= Cl
e- R_1= Me, R_2= H, R= Br
f- R_1= H, R_2= F, R= F
g- R_1= H, R_2=Br, R= Br

a) $SOCl_2$, reflux, 17h
b) 1- R-$PhNHNH_2$/toluene, 3h; 2- AcOH, reflux, 2h
c) 1- BSTFA/Me_3SiCl, N_2, 110-120 °C, 5h; 2- 1-O-acetyl-2,3,4-tri-O-benzoyl-beta-D-ribofuranose/CH_3CN, TMSOTf, r. t., 2-4h
d) 1- 0.1 N MeONa/MeOH, r. t., 16-24h; 2- sat. NH_4Cl/EtOH

Scheme 1. Synthetic route used for preparing the pyrazolo[4,3-*c*]quinolin-3-one derivatives **3a–g** and their ribonucleosides **5a–g**.

110–120°C, under nitrogen atmosphere, followed by addition of a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in acetonitrile, in the presence of trimethylsilyltrifluoromethanesulfonate (TMSOTf)[10] as a catalyst. This mixture was stirred for 2–4 h and then poured into ice-cold water. Afterwards, it was neutralized with saturated aqueous solution of sodium bicarbonate and the resulting solid was collected by filtration, washed with water (4 × 30 mL) and hexane (3 × 20 mL). Purification by column chromatography on silica gel, eluting with 20% of dichloromethane in ethyl acetate followed by crystallization from ethanol/acetone (9:1) afforded the pure protected ribonucleosides **4a–g** in 88%, 85%, 80%, 80%, 85%, 82% and 87% yields, respectively. Deprotection reaction of these nucleosides **4a–g** was carried out by

treatment with 0.1 N sodium methoxide solution (40 mL), at room temperature, for 16–24 h, followed by neutralization with saturated ethanolic ammonium chloride solution. The resulting solids were washed with cold water and ethyl ether, giving pure 1-β-D-ribofuranosylpyrazolo[4,3-*c*]quinolin-3-ones **5a–g** in 90%, 88%, 89%, 92%, 82%, 87% and 86% yields, respectively.

The structures of the new compounds are supported by 1D and 2D NMR techniques [^{1}H (Tables 1 and 2), ^{13}C (Tables 3 and 4), ^{1}Hx^{1}H-COSY, ^{1}Hx^{13}C-HETCOR ^{1}J and ^{n}J$_{CH}$, n = 2,3, nOedif], IV, UV and FABHRMS analysis. The position of glycosylation was established on the basis of cross peak between C4 and the anomeric hydrogen H1′ (^{3}J$_{CH}$) in the HETCOR spectrum of **5a**, clearly indicating that the ribosylation occurred at N5. Compounds **5a–g** afforded practically identical UV curves, confirming the glycosylation at N5 for all heterocycles. Saturation of H1′ (riboside) resulted in nuclear Overhauser enhancements of H2′, H4′, H6 and H4 signals for **4a–g**, also confirming N5 as the ribosylated nitrogen and establishing β-configuration (H4′) for all nucleosides. Furthermore, all ^{13}C NMR assignments of the ribosyl moiety of compounds **4** and **5** are very similar in their respective series. ^{13}C chemical shifts of their corresponding heterocyclic bases also show the same pattern, of which the signal in the 161 ppm region corresponds to carbon C3 of the pyrazole moiety.

BIOLOGICAL STUDIES

Antiviral Activity

The antiviral effects of pyrazolo[4,3-*c*]quinolin-3-one nucleobases and ribonucleosides on HSV-1 and vaccinia virus replication were determined as described in the Experimental section. Our results suggest that most of the nucleobases were more effective inhibitors of vaccinia virus replication than the ribonucleoside counterparts. Heterocycles **3c** and **3d** were able to inhibit VV infection near to 70%. It is important to emphasize that compounds **3c** and **3d** were not cytotoxic to host cells at the same concentration they inhibited VV infections, suggesting they may be selectively targeted to virus replication. However, these compounds showed less significant inhibitory effects on HSV-1 replication, reaching the maximal inhibitory effect around 20–30% (Table 5). The antiviral activities of the pyrazolo[3,4-*b*]pyridine derivatives against vaccinia virus have been recently described.[11] Comparatively, the antiviral agents described here are more efficient against vaccinia virus. Altogether, these results provide important information for future design of more potent antiviral pyrazolo derivatives. Furthermore, all compounds in question, synthesized by our group, probably present a common mechanism of inhibition against vaccinia virus replication, which is currently under investigation in our laboratory.

EXPERIMENTAL

General Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer

Table 1. Proton (300.00 MHz) chemical shift assignments for **4a–g** [($CDCl_3$/TMS), J (Hz)].

H	4a	4b	4c	4d	4e	4f	4g
4	8.81 (s)	8.81 (s)	8.82 (s)	8.81 (s)	8.85 (s)	8.84 (s)	8.80 (s)
6	7.49–7.40 (m)	7.63–7.51 (m)	7.49–7.31 (m)	7.50–7.31 (m)	7.65–7.53 (m)	7.48–7.33 (m)	7.86 (d, 1.5)
7	7.26 (dd, 9.0, 1.2)	7.36–7.26 (m)	7.28–7.25 (m)	7.29–7.25 (m)	7.40–7.35 (m)	–	–
8	–	–		–		7.17–7.10 (m)	7.46–7.35 (m)
9	8.26 (d, 0.9)	8.24 (d, 1.2)	8.23 (d, 1.5)	8.24–8.19 (m)	8.23 (s)	8.18–8.11 (m)	8.24 (d, 8.4)
1′	6.47 (d, 3.9)	6.48 (d, 4.2)	6.48 (d, 3.9)	6.47 (d, 3.9)	6.60 (d, 3.9)	6.44 (d, 4.8)	6.51 (d, 4.5)
2′	5.97 (dd, 5.7 e 3.9)	5.97 (dd, 5.7, 4.2)	5.97 (dd, 5.7 e 4.2)	5.97 (dd, 5.7, 4.2)	5.95 (t, 5.7)	5.94–5.85 (m)	5.90–5.83 (m)
3′	5.83 (t, 5.4)	5.83 (t, 5.7)	5.83 (t, 5.7)	5.83 (t, 5.7)	5.86 (t, 5.7)	5.94–5.85 (m)	5.90–5.83 (m)
4′	5.00–4.84 (m)	5.01–4.84 (m)	5.10–4.85 (m)	5.00–4.91 (m)	5.03–4.97 (m)	5.02–4.93 (m)	4.96–4.93 (m)
5′a	5.00–4.84 (m)	5.01–4.84 (m)	5.10–4.85 (m)	5.00–4.91 (m)	5.03–4.97 (m)	5.02–4.93 (m)	5.00 (dd, 12.0, 3.6)
5′b	5.00–4.84 (m)	5.01–4.84 (m)	5.10–4.85 (m)	4.86 (dd, 11.7, 1.8)	4.86 (dd, 13.8, 3.9)	4.84 (dd, 7.8 e 2.4)	4.85 (dd, 12.1; 2.4)
2″	8.14 (dd, 8.4, 1.5)	8.15–8.13 (m)	8.14 (dd, 8.4, 1.5)	8.13 (dd, 8.1, 1.2)	8.15 (dd, 7.9, 1.5)	8.18–8.11 (m)	8.14–8.09 (m)
	7.97 (dd, 8.4, 1.2)	7.98–7.94 (m)	7.97 (dd, 8.4, 1.2)	7.97 (dd, 8.2, 1.5)	7.98 (dd, 8.4, 1.2)	8.00–7.89 (m)	7.98 (dd, 8.4, 1.2)
	7.87 (dd, 8.4, 1.2)	7.88–7.85 (m)	7.87 (dd, 8.4, 1.2)	7.87 (dd, 8.7, 1.2)	7.90 (dd, 8.7, 1.5)		7.92 (dd, 8.3, 1.5)
3″	7.64–7.51 (m) 7.49–7.40 (m)	7.48–7.40 (m) 7.36–7.26 (m)	7.64–7.51 (m) 7.49–7.31 (m)	7.65–7.51 (m) 7.50–7.31 (m)	7.65–7.51 (m) 7.51–7.35 (m)	7.48–7.33 (m)	7.64–7.50 (m) 7.46–7.35 (m)
4″	7.64–7.51 (m)	7.63–7.51 (m)	7.64–7.51 (m)	7.65–7.51 (m)	7.65–7.51 (m	7.64–7.52 (m)	7.64–7.50 (m)
2‴	8.22 (dd, 8.7, 1.2)	8.10–8.05 (m)	8.21–8.16 (m)	8.24–8.19 (m)	8.22–8.18 (m)	8.44 (dd, 10.2, 6.3)	8.14–8.09 (m)
3‴	7.33 (t, 8.1)	7.02–6.97 (m)	7.10–7.16 (m)	7.50–7.31 (m)	7.56 (d, 9.0)	7.28–7.22 (m)	7.46–7.35 (m)
4‴	7.23–7.18 (m)	–		–		–	–
CH_3	2.46 (s)	2.46 (s)	2.47 (s)	2.49 (s)	2.49 (s)	–	–
OCH_3	–	3.85 (s)	–	–	–	–	–

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Table 2. Carbon (75.0 MHz) chemical shift assignments for **4a–g** [($CDCl_3$/TMS), J (Hz)].

C	**4a**[a]	**4b**	**4c**	**4d** (CD_2Cl_2)	**4e** (CD_2Cl_2)	**4f**	**4g**
3	161.8	161.4	161.6	162.0	162.1	161.1	161.3
3a	108.7	108.5	108.4	108.8	108.8	109.3	109.0
4	135.6	135.6	135.7	135.7	135.7	136.1	135.7
5a	132.9	133.1 or 132.8	132.8	133.5	133.5	136.2 (J = 9.8)	135.8
6	115.7	115.7	115.6	116.0	116.1	103.2 (J = 27.8)	119.0
7	131.4	131.2	131.3	131.8	131.9 or 131.7	163.0 (J = 249.0)	124.1
8	136.9	136.6	136.8	137.4	137.5	114.6 (J = 22.3)	d
9	123.5	123.5	123.4	123.3	123.7	125.7 (J = 9.8)	124.8
9a	120.1	120.5	120.0	120.3	120.3	116.3 (J = 2.7)	118.6
9b	142.4	142.1	142.3	142.9	143.0	141.6	141.7
1′	91.3	91.4	91.3	91.0	91.1	90.8	90.1
2′	74.0	74.1	74.2	74.7	74.8	74.4	75.0
3′	69.9	70.0	69.9	70.2	70.3	70.3	70.6
4′	80.4	80.4	80.4	80.9	81.0	80.9	81.4

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5′	62.6	62.7	62.6	62.9	62.9	62.8	63.0
1″	128.8, 128.1, 127.9	128.8, 128.2, 127.9	128.8, 128.1, 127.9	b	129.3, 128.6, 128.3	128.7, 128.1 127.7	128.8, 128.2, 127.8
2″	129.7, 129.6	129.7; 129.6	129.7; 129.6	129.9, 129.8	130.0, 129.9	129.6, 129.5	d
3″	a	128.6, 128.5, 128.4	128.5, 128,3	c	128.9, 128.8, 128.7	128.5, 128.4, 128.3	128.6, 128.5, 128.4
4″	133.9, 133.6, 133.4	133.9, 133.6, 133.4	133.9, 133.6, 133.4	134.2, 133.9, 133.7	134.2, 133.9, 133.7	133.9, 133.6, 133.3	133.9, 133.6, 133.3
1‴	139.7	133.1 or 132.8	135,9 (J = 2.5)	139.0	139.5	135.6 (2.7)	138.5
2‴	119.6	121.6	121,8 (J = 8.0)	120.4	120.8	120.9 (J = 7.6)	120.4
3‴	a	113.8	115,1 (J = 22.6)	c	131.9 or 131.7	115.0 (J = 22.3)	131.4
4’’’	124.5	156.7	156,5 (J = 242.3)	b	117.0	159.6 (J = 259.0)	117.2
O–C	166.0	166.0	165.9	166.2	166.2	166.0	166.0
O–C	164.8	164.8	164.8	165.1	165.1	164.8	164.8
O–C	164.5	164.5	164.5	164.8	164.9	164.7	164.4
OCH_3	–	55.3	–	–	–	–	–
CH_3	20.8	20.8	20.7	20.9	20.9	–	–

a: 128.6, 128.5 or 128.3; b: 129.2, 129.2, 128.3; c: 128.9, 128.8, 128.7 or 128.6; d: 129.8, 129.7, 129.6 or 129.5; e: 129.8, 129.7, 129.6; f: 128.6, 128.4.

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Table 3. Proton (300.00 MHz) chemical shift assignments for **5a–g** [(DMSO-d6/TMS), J (Hz)].

H	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**
4	9.33 (s)	9.33 (s)	9.37 (s)	9.37 (s)	9.38 (s)	9.40 (s)	9.36 (s)
6	7.90 (d, 8.7)	7.90 (d, 8.7)	7.90–7.88 (m)	7.90 (d, 9.0)	7.90 (d, 9.0)	7.84 (d, 11.7)	8.17 (s)
7	7.71 (dd, 9.0, 2,1)	7.70 (d, 8.7)	7.72–7.69 (m)	7.71 (d, 9.0)	7.76–7.70 (m)	–	–
8	–	–	–	–	–	8.64 (d, 8.4)	7.90 (d, 8.7)
9	8.24 (bs)	8.23 (bs)	8.23 (bs)	8.23 (bs)	8.24 (bs)	8.47 (dd, 8.7, 6.3)	8.32 (d, 8.4)
1′	6.24 (d, 2.4)	6.25 (d, 2.4)	6.25 (sl)	6.25 (d, 2.4)	6.25 (d, 1.8)	6.21 (d, 2.7)	6.24 (d, 2.1)
2′	4.32–4.20 (m)	4.30–4.20 (m)	4.31–4.20 (m)	4.32–4.20 (m)	4.30–4.20 (m)	4.34–4.21(m)	4.35–4.30 (m)
3′	4.32–4.20 (m)	4.30–4.20 (m)	4.31–4.20 (m)	4.32–4.20 (m)	4.30–4.20 (m)	4.34–4.21 (m)	4.26–4.20 (m)
4′	4.32–4.20 (m)	4.30–4.20 (m)	4.31–4.20 (m)	4.32–4.20 (m)	4.30–4.20 (m)	4.34–4.21 (m)	4.26–4.20 (m)
5′a	4.02 (dd, 13.0, 3.9)	4.06–3.88 (m)	4.04–4.00 (m)	4.07–4.00 (m)	4.02 (dd, 3.9, 11.1)	4.05–4.00 (m)	4.00 (dd, 9.9; 0.9)
5′b	3.82 (dd, 13.0, 3.9)	3.88–3.78 (m)	3.85–3.80 (m)	3.85–3.80 (m)	3.82 (dd, 3.9, 11.1)	3.88–3.81 (m)	3.82 (dd, 9.9, 0.9)
OH-2′	5.97 (d, 5.1)	6.01 (d, 5.4)	6.02 (d, 4.5)	6.02 (d, 5.1)	6.04 (d, 4.8)	6.06 (d, 5.1)	6.04 (d, 5.1)
OH-3′	5.37–5.34 (m)	5.40–5.38 (m)	5.41–5.38 (m)	5.40–5.38 (m)	5.41–5.38(m)	5.40–5.37 (m)	5.43–5.41 (m)
OH-5′	5.50 (t, 4.2	5.53 (t, 4.8)	5.58–5.54 (m)	5.55 (t, 4.5)	5.55 (t, 4.5)	5.56 (t, 4.5)	5.55 (t, 4.2
2″	8.32 (dd, 7.5, 1.2)	8.20–8.17 (m)	8.36–8.28 (m)	8.36 (d, 9.0)	8.31 (d, 9.0)	8.30 (dd, 9.0, 5.1)	8.27 (d, 9.0)
3″	7.56 (t, 7.5)	7.13 (d, 9.0)	7.44–7.37 (m)	7.62 (d, 9.0)	7.76–7.70 (m)	7.41 (t, 8.4)	7.73 (d, 9.0)
4″	7.29 (t, 7.5)	–	–	–	–	–	–
CH_3	2.60 (s)	2.62 (s)	2.62 (s)	2.62 (s)	2.62 (s)	–	–
OCH_3	–	3.90 (m)	–	–	–	–	–

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Table 4. Carbon (75.0 MHz) chemical shift assignments for **5a–g** [(DMSO-d6/TMS), J (Hz)].

C	5a	5b	5c	5d	5e	5f	5g
3	161.6	161.1	161.5	161.8	161.8	161.8	161.5
3a	106.3	106.3	106.1	106.0	106.0	107.5	106.8
4	137.2	137.1	137.5	137.5	137.5	138.4	138.4
5a	133.4	133.6 or 133.3	133.4	133.4	133.4	137.0	136.5
6	117.5	117.5	117.6	117.6	117.6	105.3 (J = 27.6)	120.4 or 120.3
7	131.5	131.5	131.7	131.7	131.6 or 131.7	163.0 (J = 300.0)	123.7
8	136.4	136.5	136.7	136.7	136.7	115.4 (J = 22.4)	129.7
9	122.2	122.2	122.3	122.3	122.3	125.0	124.5
9a	119.	119.5	119.5	119.4	119.3	116.8	118.5
9b	142.5	142.1	142.7	143.0	142.9	142.9	142.5
1′	92.7	92.9	93.0	93.0	93.0	93.1	92.9
2′	75.0	75.1	75.1	75.2	75.2	75.0	75.1
3′	68.6	68.5	68.5	68.5	68.4	68.6	68.7
4′	84.7	84.6	84.6	84.6	84.6	84.9	84.9
5′	59.5	59.4	59.4	59.4	59.3	59.4	59.5
1″	133.4	133.6 or 133.3	136.6 (J = 2.5)	139.0	139.4.	139.0	139.1
2″	118.6	120.5	120.5 (J = 7.3)	120.0	120.3	120.5	120.4 or 120.3
3″	128.6	113.9	115.5 (J = 22.2)	128.9	131.6 or 131.7	116.0 (J = 22.5)	131.7
4″	124.0	156.1	158.8 (J = 239.4)	127.9	116.0	159.7 (J = 293.9)	116.1
OCH_3	–	55.4	–	–	–	–	–
CH_3	20.5	20.7	20.7	20.7	20.7	–	–

1420 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Ultraviolet (UV) spectra were obtained on a Shimadzu spectrophotometer; λ are in nm and ε in $mol^{-1}cm^{-1}$. High resolution mass spectra (FABHRMS) were recorded in a 3-nitrobenzylalcohol matrix in the positive ion mode on a VG ZAB-E mass spectrometer, by the Department of Chemistry and Biochemistry, University of Oklahoma. NMR spectra were recorded on a Varian Unity Plus 300 spectrometer operating at 300.00 MHz (^1H) and 75.0 MHz (^{13}C), in specified solvents. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Proton and carbon spectra were typically obtained at room temperature. The two dimensional experiments were acquired using standard Varian Associates automated programs for data acquisition and processing.

General Procedures for the Syntheses of: 2-Phenyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-ones (3a–g); 2-Phenyl-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-ones (4a–g) and 2-Phenyl-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-ones (5a–g). The quinolone derivatives **1a–g** were

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Table 5. Antiviral activity of pyrazolo[4,3-*c*]quinolin-3-ones (**3a**–**3f**) and ribonucleoside derivatives (**5a**–**5f**).

	% of inhibition of virus yield	
Compounds 100 μM	HSV-1	VV
3a	20	47
3b	22	33
3c	31	68
3d	27	70
3e	27	42
3f	26	55
5a	20	30
5b	30	48
5c	15	18
5d	35	8
5e	25	10
5f	35	9

prepared by treating the appropriate aniline with diethyl ethoxymethylenemalonate to obtain the enamine derivatives that were then cyclized in refluxing Dowtherm A.[1] These quinolones (13 mmol) were refluxed in thionyl chloride (20 mL), for 17 h, affording the corresponding chloro-derivatives **2a**–**g**.[3,4] Reaction of **2a**–**g** (4 mmol) with the desired phenylhydrazine (8 mmol) in toluene (30 mL), for 3 h, followed by a 2 h-reflux in acetic acid gave the corresponding 2-phenylpyrazolo[4,3-*c*]quinolin-3-ones **3a**–**g**. Heating **3a**–**g** (1 mmol) with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (3.0 mL)[7–9] containing 1% of TMSCl at 110–120°C, under nitrogen atmosphere, for 5 h, followed by addition of a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.1 mmol) in 15 mL of acetonitrile, in the presence of trimethylsilyltrifluoromethanesulfonate (TMSOTf) (1.2 mmol in 5 mL of acetonitrile)[10] as a catalyst, at room temperature, and subsequent stirring for 2–4 h led to the crude product, which was poured into ice-cold water (20 mL). After neutralization with saturated aqueous solution of sodium bicarbonate, the resulting solid was collected by filtration, washed with water (4 × 30 mL) and hexane (3 × 20 mL). Purification by column chromatography on silica gel, eluting with 20% of dichloromethane in ethyl acetate followed by crystallization from ethanol/acetone (9:1) afforded the pure protected ribonucleosides **4a**–**g**. Deprotection reactions of these nucleosides **4a–g** (0.5 mmol) were carried out by treatment with 0.1 N sodium methoxide solution (40 mL), at room temperature, for 16–24 h, followed by neutralization with saturated ethanolic ammonium chloride solution, affording solid products which were washed with cold water and ethyl ether, giving pure 1-β-D-ribofuranosylpyrazolo[4,3-*c*]quinolin-3-ones **5a–g**.

4a: 8-Methyl-2-phenyl-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 88%, m.p. 201°C; FABHRMS m/z calcd. for $C_{43}H_{34}N_3O_8$ $(M + H)^+$ 720.2343, found 720.2346; IR 3060, 1720, 1630, 1260; UV λ_{max} ($CHCl_3$) 244 (ε 33,018), 411 (ε 5,312); ^{1}H NMR (Table 1); ^{13}C NMR (Table 2).

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4b: 8-Methyl-2-(4-methoxyphenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 85%, m.p. 248–249°C; FABHRMS m/z calcd. for $C_{44}H_{36}N_3O_9$ $(M + H)^+$ 750.2449, found 750.2451; IR 3060, 1715, 1630, 1260; UV λ_{max} ($CHCl_3$) 243 (ε 18,311), 411 (ε 2,711); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

4c: 8-Methyl-2-(4-fluorophenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 80%, m.p. 225–226°C; FABHRMS m/z calcd. for $C_{43}H_{33}N_3O_8F$ $(M + H)^+$ 738.2250, found 738.2251; IR 3060, 1720, 1630, 1260; UV λ_{max} ($CHCl_3$) 243 (ε 21,731), 418 (ε 2,934); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

4d: 8-Methyl-2-(4-chlorophenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 80%, m.p. 237–238°C; FABHRMS m/z calcd. for $C_{43}H_{33}N_3O_8{}^{37}Cl$ $(M + H)^+$ 756.1898, found 756.1926; IR 3050, 1720, 1635, 1260; UV λ_{max} ($CHCl_3$) 243 (ε 13,233), 411 (ε 1,718); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

4e: 8-Methyl-2-(4-bromophenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 85%, m.p. 238°C; FABHRMS m/z calcd. for $C_{43}H_{33}N_3O_8{}^{81}Br$ $(M + H)^+$ 798.1550, found 798.1551; IR 2970, 1720, 1635, 1260; UV λ_{max} ($CHCl_3$) 243 (ε 61,700), 411 (ε 8,660); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

4f: 7-Fluoro-2-(4-fluorophenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 82%, m.p. 212°C; FABHRMS calcd. for 798.1550, found 798.1551; IR 2970, 1720, 1635, 1260; UV λ_{max} ($CHCl_3$) 285 (ε 55,384), 421 (ε 7,024); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

4g: 7-Bromo-2-(4-bromophenyl)-5-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 87%, m.p. 227°C; FABHRMS m/z calcd. for $C_{42}H_{30}N_3O_8{}^{81}Br_2$ $(M + H)^+$ 866.0357, found 866.0358; IR 3060, 1725, 1615, 1260; UV λ_{max} ($CHCl_3$) 296 (ε 23,726), 421 (ε 2,975); ^1H NMR (Table 1); ^{13}C NMR (Table 2).

5a: 8-Methyl-2-phenyl-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 90%, m.p. 282°C; FABHRMS m/z calcd. for $C_{22}H_{22}N_3O_5$ $(M + H)^+$ 408.1560, found 408.1559; IR 3660–2700, 1630, 1260; UV λ_{max} (CH_3OH) 393 (ε 5,392), 223 (ε 30,400); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

5b: 8-Methyl-2-(4-methoxyphenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 88%, m.p. 258–259°C; FABHRMS m/z calcd. for $C_{23}H_{24}N_3O_5$ $(M + H)^+$ 438. 1663, found 438.1664; IR 3620–2800, 1625; UV λ_{max} (CH_3OH) 391 (ε 5,500) 225 (ε 43,800); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

5c. 8-Methyl-2-(4-fluorophenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 89%, m.p. 274°C; FABHRMS m/z calcd. for $C_{22}H_{21}N_3O_5F$ $(M + H)^+$426.1463 found 426.1465; IR 3620–2600, 1630; UV λ_{max} (CH_3 OH) 391 (ε 4,262), 223 (ε 24,900); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

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5d: 8-Methyl-2-(4-chlorophenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 92%, m.p. 250°C; FABHRMS m/z calcd. for $C_{22}H_{21}N_3O_5{}^{35}Cl$ $(M + H)^+$ 444.1168, found 444.1169, $C_{22}H_{21}N_3O_5{}^{37}Cl$ $(M + H)^+$ 444.1141, found 444.1140; IR 3650–2800, 1610; UV λ_{max} (CH_3OH) 393 (ε 3,243), 225 (ε 31,500); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

5e: 8-Methyl-2-(4-bromophenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 82%, m.p. 256°C; FABHRMS m/z calcd. for $C_{22}H_{21}N_3O_5{}^{79}Br$ $(M + H)^+$ 486.0763, found 486.0664, $C_{22}H_{21}N_3O_5{}^{81}Br$ $(M + H)^+$ 488.0644, found 488.0643; IR 3630–2800, 1625; UV λ_{max} (CH_3OH) 389 (ε 3,724), 225 (ε 23,797); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

5f: 7-Fluoro-2-(4-fluorophenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-c]quinolin-3(5H)-one- 87%, m.p. 241–242°C; MS m/z 419 (5); IR 3660–2800, 1620; UV λ_{max} (CH_3 OH) 391 (ε 8,603), 223 (ε 48,168); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

5g: 7-Bromo-2-(4-bromophenyl)-5-β-D-ribofuranosyl-2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one- 86%, mp. 258°C; FABHRMS m/z calcd. for $C_{21}H_{18}N_3O_5{}^{79}Br_2$ $(M + H)^+$ 549.9612, found 549.9613, $C_{21}H_{18}N_3O_5{}^{79}Br_2{}^{81}Br_2$ $(M + H)^+$ 551.9691, found 551.9592; UV λ_{max} (CH_3OH) 388 (ε 6,450), 226 (ε 34,919); ^1H NMR (Table 3); ^{13}C NMR (Table 4).

Cells and Viruses: BSC-40 and Vero, epithelial-derived cell lines from the African green monkey kidney, were obtained from the American Type Culture Collection and were grown in monolayer cultures in Dulbecco's modified Eagle's (Gibco laboratories) containing 2% heated-inactivated fetal bovine serum (purchased from Fazenda Pig), 8% calf serum (purchased from Centro Pan-Americano de Febre Aftosa), 2.25% sodium bicarbonate, 500 U/mL penicilin, 100 μg/mL streptomycin, 50 μg/mL gentamycin, 2.5 μg/mL amphotericin B. Cell cultures were incubated at 37°C in humidified air atmosphere containing 5% CO_2.

The vaccinia virus (WR strain) was obtained from the American Type Culture Collection and was routinely propagated and titrated in confluent BSC-40 cells, as described by Dâmaso and Moussatché.[12] Herpes simplex virus type 1 (HSV-1) was kindly provided by Marcia Wigg (Universidade Federal do Rio de Janeiro, Brasil) and was routinely propagated in Vero cells. Virus stocks were stored at −70°C until use.

Tissue Culture Inhibitory Dose ($TCID_{50}$) for HSV-1 Titration: Virus infectivity was measured by a dilution method using 96-well microtitre plate and expressed as 50% tissue culture infections dose ($TCID_{50}$) according to Reed and Muench.[13] Sub-confluent VERO cells, grown in 96 well plates (10^6 cells/well), were infected with HSV-1 at 1 PFU (plaque forming unit)/cell for 120 minutes at 37°C in 100 μL of DMEM). After this period, virus inoculum was replaced by a culture medium, containing pyrazolo[4,3-*c*]quinolin-3-one derivatives or their respective ribonucleosides at the concentration of 100 μM. Control cultures were incubated with media without compounds. After 72 hours post-infection, the culture medium was harvested and the virus titer of each sample was determined in terms of the 50 % tissue culture dose (TCID 50/mL) by endpoint dilution.

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Yield Reduction Assay for Vaccinia Virus Titration: Sub-confluent BSC-40 cells, grown in 6-well plates (10^6 cells/mL), were infected with 2.5 PFU of VV per cell. After 20 minutes of virus adsorption at 37°C in 200 µl of DMEM, virus inoculum was replaced by culture medium, containing or not either pyrazolo[4,3-*c*]quinolin-3-one heterocycle moieties or their respective ribonucleosides at the concentration of 100 µM. Infection proceeded for 12 hours at 37°C in humidified air containing 5% CO_2, when the culture medium was aspirated and cell monolayers were harvested in 500 µL of 1 mM Tris-HCL pH 9.0 and submitted to two cycles of freezing thawing for homogenization. Virus titer of each sample was determined by fixing and staining infected cell monolayers with PBS containing 0.1% crystal violet, 3.7% formaldehyde, for 30 minutes. Afterwards, cell monolayers were thoroughly washed with water and virus plaque number was determined. The yield reduction was determined by using the virus yield obtained in the absence of drugs as a reference.

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