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Synthesis and anti-HSV-1 activity of quinolonic acyclovir analogues

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Abstract—Several 1-[(2-hydroxy-ethoxy)methyl]-3-carbethoxy-4(1*H*)quinolones (**2a**–**l**) and 1-[(2-hydroxy-ethoxy)methyl]-4(1*H*)quinolone-3-carboxylic acids (**3a**–**j** and **3l**) were synthesized and **2a**–**j**, **2l** and **3a**–**j**, **3l** were evaluated against herpes simplex virus type 1 (HSV-1), employing a one-pot reaction: silylation of the desired quinolone (BSTFA 1% TMCS) followed by equimolar amount addition of 1,3-dioxolane, chlorotrimethylsilane and KI, at room temperature. The acyclonucleosides **2a**–**l** were obtained in 40–77% yields. The esters **2a**–**j** and **2l** were subsequently converted into the corresponding hydroxyacids **3** in 40–70% yields. Attempts of hydrolysis of **2k** produced only a mixture of degradation products. Antiviral activity of **2** and **3** on HSV-1 virus infection was assessed by the virus yield assay. Except for compounds **2i** and **3e**, the acyclonucleosides were found to reduce the virus yield by 70–99% at the concentration of 50 μM, being the acids, in general, more effective inhibitors than their corresponding esters. Compounds **3j** and **2d** exhibited antiviral activity against HSV-1 virus with EC_{50} of 0.7 ± 0.04 and 0.8 ± 0.09 μM, respectively. Both compounds were not toxic towards the Vero cell line.
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Herpes Simplex Virus type 1 (HSV-1) is a large enveloped virus containing double-stranded DNA genomes of approximately 152 kb in size. HSV-1 is the primary cause of oral and facial lesions in humans. Infection by HSV-1 usually begins on the skin or mucosal epithelium and subsequently spreads to the sensorial ganglia, whose nerve process contacts the primary site of infection. Once inside these neurons of the sensorial ganglia, the virus may continue to produce infection in certain cells expressing three viral gene classes (IE, immediate early; E, early; L, late) or the virus may enter a state of latency characterized by the absence of lytic gene transcription.[1] Recently, there have been considerable advances in the chemotherapy of virus infections. Nevertheless, with the increase in the use of antiviral drugs, a development of resistance to antivirals has been observed. In the chemotherapy of herpes simplex virus infections, acyclovir (**1**) is used clinically as an anti-herpes drug. However, herpes viruses may sometimes acquire resistance to acyclovir in immuno-compromised patients.[2] Many research approaches are currently aimed at developing new types of antiviral agents that have a wide range of efficacy without serious adverse effects and are potent against viral strains resistant to current antiviral agents. The search for more effective antiviral agents has so far developed only a few compounds that have reached prominence at clinical level so far. Acyclic nucleosides are of interest because of the antiviral activity of a large number of this class of compounds.[3–7] Up to now, there have only been a few reports regarding the synthesis of quinolone acyclonucleoside derivatives.[8] We describe herein the extension of Ubasawa[9] one-pot synthesis method of acyclonucleosides for the synthesis of 1-[(2-hydroxy-ethoxy)methyl]-3-carbethoxy-4(1*H*)quinolones **2a**–**l** and 1-[(2-hydroxy-ethoxy)methyl]-4(1*H*)quinolone-3-carboxylic acids **3a**–**j** and **3l**, establishing now an efficient and generally applicable methodology for the synthesis of acyclovir quinolonic analogues. We have also studied the effects of these acyconucleosides on herpes simplex virus type 1 replication in Vero cells.

Keywords: Acyclonucleoside; Quinolone; HSV-1.
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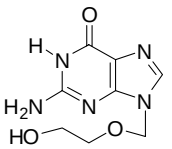


1- Acyclovir

The synthesis of 1-[(2-hydroxy-ethoxy)methyl]-3-carbethoxy-4(1*H*)quinolones **2** and 1-[(2-hydroxy-ethoxy)methyl]-4(1*H*)quinolone-3-carboxylic acids **3** was initiated from substituted 4-(1H) quinolones **4**, prepared in good yields (60–88%) employing the treatment of appropriate meta- and *para*-substituted anilines **5** with diethyl ethoxymethylenemalonate to obtain the enamine-type derivatives **6** in 74–96% yields, which were then cyclized in refluxing Dowtherm A (Scheme 1).[10]

Acyclonucleosides **2a**–**l** were prepared by previous silylation of the quinolones by heating with bis(trimethylsilyl)trifluoroacetamide (BSTFA)[11–13] containing 1% of trimethylchlorosilane (TMCS), in acetonitrile, under reflux, followed by addition of equimolar amount of 1,3-dioxolane, chlorotrimethylsilane, and potassium iodide, at room temperature. Afterwards, the reaction was quenched by addition of methanol followed by neutralization with solid sodium bicarbonate (Scheme 1). Purification by crystallization from ethyl alcohol/methylene chloride mixture (1:1) led to the desired pure compounds **2a**–**l** in 75%, 45%, 70%, 58%, 72%, 77%, 47%, 57%, 68%, 50%, 40% and 70% yields, respectively. It is worth noting that the isolated nucleosides maintain the carboxylic ethyl ester group at carbon C^3 of the quinolonic nucleus enabling further functionalization of the free hydroxilic group of the acyclic unity, if desired.

The treatment of the compounds **2a**–**j** and **2l** with 0.1 N ethanolic sodium hydroxide solution, at room temperature, followed by neutralization with Dowex 50 H^+ produced the 3-carboxylic acyclonucleoside acids in 65%, 58%, 42%, 62%, 40%, 57%, 50%, 56%, 64%, 41% (**3a**–**j**, respectively) and 70% (**3l**) yields (Scheme 1).

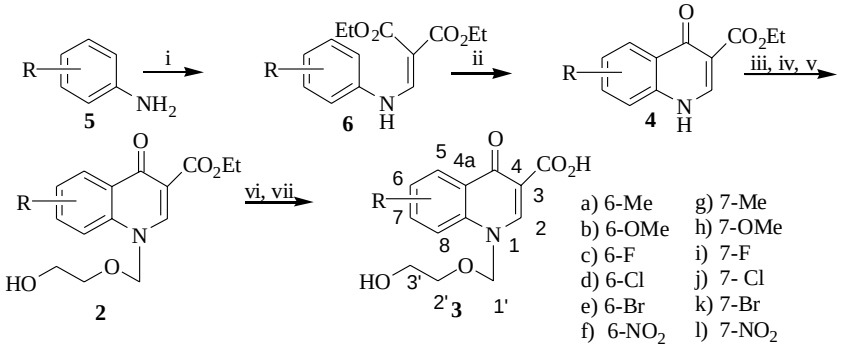
Hydrolysis reaction of **2k** produced only a mixture of degradation products.

Attempts to introduce the acyclic chain into the heterocycle by silylation of the quinolones in a refluxing mixture of hexamethyldisilazane (HMDS) and ammonium sulfate or in refluxing BSTFA/TMCS using acetonitrile as solvent, and subsequent treatment with acetoxyethyl-acetoxymethyl ether under trimethylsilyltrifluoromethanesulfonate (TMSOTf) catalysis were unsuccessful.

The structures of the new compounds are supported by spectroscopic analysis.

NMR spectra were recorded on a Varian Unity Plus 300 spectrometer operating at 300.00 MHz (^{1}H) and 75.0 MHz (^{13}C). Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (Supplementary data: files a–d). 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 preparation of these acyclonucleosides, along with analytical characterization for **2d** and **3d**, are given.[14]

The antiviral effect of quinolone acyclonucleoside carboxylic acids **3** and their corresponding esters **2** on HSV-1 virus replication was determined. Our results show that except for compounds **2i** and **3e**, carboxylic acids **3** and the esters **2** were found to reduce the virus yield in 70–99% at the concentration of 50 μM, being the acids, in general, more effective inhibitors than their corresponding esters (Table 1). Compounds with nearly the same antiviral effects were evaluated for cytotoxicity in Vero cells. EC_{50} and the selectivity index (SI) were determined in parallel. Several of the new compounds prevented the cytopathic effect of HSV-1 in Vero cells, at micromolar concentrations, and were minimally toxic to Vero cells resulting in a good selectivity index. The MTT assay indicated that compounds **3d**, **3j** and **2d** exhibited a low cytotoxicity, CC_{50} 1400, 1200 and 1250 μM, respectively. Trypan blue and MTT showed similar results (data not showed). Acyclovir (**1**) results have been included for comparison purposes. Compounds **3j** and **2d** were the most effective anti-HSV-1 derivatives and presented a 1.5- and 1.3-fold increase in their antiviral activity in relation to acyclovir (**1**) (Table 2). The



Scheme 1. Synthesis of quinolonic acyclonucleosides **2** and **3**. Reagents and conditions: (i) EtOH, diethyl ethoxymethyleremalonate, reflux; (ii) Dowtherm A, reflux; (iii) BSTFA/TMSCl, CH_3CN, reflux; (iv) 1,3-dioxolane/TMSCl/KI, rt; (v) $NaHCO_3$; (vi) NaOEt//EtOH 0.6 M; (vii) Dowex-H^+.

Table 1. Antiviral activity of quinolonic acyclonucleosides **2a–j**, **2l**, **3a–j** and **3l**

Compound	R-C^6	R-C^7	% of inhibition of virus yield (HSV-1)
3a	Me	H	93 ± 1
3b	O-Me	H	89 ± 2
3c	F	H	70 ± 2
3d	Cl	H	98 ± 1
3e	Br	H	58 ± 1
3f	NO_2	H	70 ± 3
3g	H	Me	79 ± 5
3h	H	O-Me	79 ± 3
3i	H	F	97 ± 7
3j	H	Cl	96 ± 4
3l	H	NO_2	77 ± 8
2a	Me	H	82 ± 1
2b	O-Me	H	99 ± 1
2c	F	H	97 ± 2
2d	Cl	H	97 ± 1
2e	Br	H	84 ± 5
2f	NO_2	H	95 ± 1
2g	H	Me	77 ± 1
2h	H	O-Me	89 ± 1
2i	H	F	65 ± 2
2j	H	Cl	99 ± 1
2l	H	NO_2	77 ± 1
ACV	—	—	96 ± 1

The experimental concentrations of quinolonic acyclonucleosides **2a–j**, **2l**, **3a–j** and **3l** were 50 μM and ACV concentration was 10 μM. Results are presented as means of triplicate experiments. ACV-acyclovir has been included for comparison purposes.

Table 2. Anti-HSV-1 activity, cytotoxicity and selectivity index in Vero cells for some quinolonic acyclonucleosides **2** and **3**

Compound	R-C^6	R-C^7	EC_{50}[a] (μM)	CC_{50}[b] (μM)	S.I.[c]
3a	Me	H	2.2 ± 0.02	>500	227
3d	Cl	H	1.1 ± 0.03	1400 ± 238	1273
3i	H	F	1.4 ± 0.8	>500	357
3j	H	Cl	0.7 ± 0.04	1200 ± 205	1714
2b	O-Me	H	3.2 ± 1.1	>500	156
2c	F	H	2.3 ± 0.9	>500	217
2d	Cl	H	0.8 ± 0.09	1250 ± 179	1562
2f	NO_2	H	1.5 ± 0.6	960 ± 128	640
2j	H	Cl	0.87 ± 0.1	1196 ± 30	1375
ACV	—	—	1.09 ± 0.25	960 ± 156	880

[a] 50% Effective concentration or concentration required to inhibit HSV-1 virus yield.
[b] 50% Cytotoxic concentration or concentration required to reduce the viability of host cells by 50%.
[c] Selective index (CC_{50}/EC_{50}).

quinolone nucleobases have not inhibited HSV-1 virus replication (data not showed). Additionally, it seems that the position and the nature of the substituent on the quinolone ring, C^6 or C^7 carbons, are more important than the type of the substituent at C^3—a carboxylic acid or an ethylcarboxylate group.

In conclusion, a new series of acyclovir analogues (**2** and **3**) was synthesized and some of them are potent anti-HSV-1 agents.

The mechanism of antiviral activity of these compounds is under investigation. For the biological assays, Vero, African green monkey kidney cells (obtained from the American Type Culture Collection), were grown in Dulbecco's modified Eagle's medium (DMEM-Gibco Laboratories) supplemented with 2% heat-inactivated foetal bovine serum (purchased from Fazenda Pig), 8% calf serum (purchased from Centro Pan- Americano de Febre Aftosa), 2.25% sodium bicarbonate, 500 U/mL penicillin, 100 μg/mL streptomycin and 2.5 μg/mL amphotericin B. Herpes simplex virus type 1 (HSV-1), strain ACR-29,[15] 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. Acyclovir (**1**) was purchased from Sigma (A 4669). It was dissolved in sterile deionized water and further diluted in culture medium. MTT (3-(4,5-dimethyltrazol-2yl)-2,5-diphenyl tetrazolium bromide) was purchased from Sigma. Virus infectivity was measured by a dilution method using a 96-well microtitre plate and expressed as 50% tissue culture infections dose ($TCID_{50}$) according to Reed and Muench.[16] Cells grown in 96-well microtitre were inoculated with virus at input 1 PFU (plaque-forming unit)/cell for 2 h at 37 °C. After virus adsorption, virus inoculum was replaced by a culture medium containing quinolone acyclonucleobases carboxylic acid and their correspondent esters at the concentration of 50 μM. Control cultures were incubated with media without compounds. After 3 days of incubation at 37 °C in 5% CO_2 atmosphere, the culture medium was harvested and the virus titre of each sample was determined in terms of 50% tissue culture dose (TCID 50/mL) by endpoint dilution.

The cytotoxicity of the compounds was tested in Vero cells using two methods, MTT (3-(4,5-diethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and trypan blue dye exclusion assay. Monolayers of uninfected cells were incubated with culture medium containing different concentrations of compounds for 72 h at 37 °C. The medium was then removed, the cells trypsinized and viable cells counted by trypan blue dye exclusion test.[17] The 50% cytotoxic concentration (CC_{50}) was calculated by regression analysis of the dose–response curves generated from these data. In the second method, monolayer of Vero cells in 96-multiwell plates were incubated with MTT (5 μg/mL) at 37 °C for 4 h. After this period, SDS 10% and 0.01 N HCl were added to each well and incubated overnight. The plates were read using an automatic plate reader with a 540 nm test wavelength and a 690 nm reference wavelength.[18] Plaque reduction assay was performed utilizing Vero cells at a density of 3×10^5 infected with various dilutions of the supernatant from a yield reduction assay for 1 h at 37 °C and 5% CO_2. After adsorption, the plates were washed and the medium was replaced with DMEM containing methylcellulose 1% and fetal bovine serum 5%. After incubation for 72 h, the monolayers were fixed with 1% formaldehyde in PBS, methylcellulose removed, and cell stained with a 0.1% solution of crystal violet in 70% methanol. The virus yield assay was performed as follows. Confluent Vero cells were washed with PBS and infected with HSV-1 at moi of 1 pfu/cell for 1 h at 37 °C. The infected cells were washed with PBS and

covered with a culture medium containing either no compounds or a different concentration of compounds. 20 h after adsorption, cells were lysed by freezing and thawing (three times), and the supernatant consisting of culture medium and lysed cells was obtained by centrifugation at 400*g* for 10 min at 4 °C. Virus titre was determined by the plaque assay in Vero cells as described above. Data were statistically analyzed by Student's *t* test for a significance level of $p < 0.05$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.10.111.

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14. General Procedures for the syntheses of 1-[(2-hydroxy-ethoxy)methyl]-3-carbethoxy-4(*1H*)quinolones **2a**–**l** and 1-[(2-hydroxy-ethoxy)methyl]-4(1*H*)quinolone-3-carboxylic acids **3a**–**j** and **3l**: The quinolone derivatives **4** were prepared by treating the appropriate aniline with diethyl ethoxymethylenemalonate to obtain the enamine derivatives that were then cyclized in refluxing Dowtherm A. These quinolones (3 mmol) were refluxed in bis(trimethylsilyl)trifluoroacetamide (BSTFA) (6.75 mmol) containing 1% TMSCl, in 10 mL acetonitrile, under nitrogen atmosphere, for 4 h, followed by addition of potassium iodide (3 mmol), 1,3-dioxolane (3 mmol) and TMSCl (3 mmol). The resulting mixture was stirred for 24 h at room temperature. Afterwards, it was poured into a mixture of methyl alcohol/water (20 mL:10 mL), followed by addition of solid sodium bicarbonate (3 mmol) and subsequent stirring for 10 min, leading to the crude products, which were purified by crystallization from a ethyl alcohol/methylene chloride mixture (1:1), giving the pure acyclonucleosides **2a**–**l**. Reaction of these nucleosides **2a**–**j** and **2l** (1 mmol) with 30 mL of 0.7 N ethanolic sodium hydroxide solution, at room temperature, for 5 h, followed by neutralization with Dowex 50-(acid form), gave pure 1-[(2-hydroxy-ethoxy)methyl]-4(1*H*)quinolone-3-carboxylic acids **3a**–**j** and **3l**, respectively. Analytical data for compounds **2d** and **3j** are showed. NMR spectra were recorded on a Varian Unity Plus 300 spectrometer operating at 300.00 MHz (^{1}H) and 75.0 MHz (^{13}C) in $CDCl_3$, for **2d** and DMSO-d_6 for **3j**, at room temperature: **2d**—1-[(2-hydroxy-ethoxy)methyl]-3-carbethoxy-6-chloro-4(1*H*)quinolone 58%, mp 146–147 °C; IR (KBr) cm^{-1} 3378, 1722, 1618; ^{1}H NMR δ 8.93 (s, H^2), 8.27 (d, J = 2.1 Hz, H^5), 8.00 (d, J = 8.7 Hz, H^8), 7.95 (dd, J = 9.0 and 2.4, H^7), 5.89 (s, H$^{1'}$), 4.76 (t, J = 5, 1 Hz, O*H*), 4.37 (q, J = 7.2 Hz, CH_2), 3.64–3.68 (m, H$^{3'}$), 3.59–3.62 (m, H$^{4'}$), 1.42 (t, J = 7.2 Hz, CH_3). ^{13}C NMR δ 172.0 (C^4), 164.0 (*C*OOEt), 149.1 (C^2)$^-$, 137.2 (C^{8a}), 132.3 (C^7), 129.9 (C^6), 128.7 (C^{4a}), 124.7 (C^5), 120.2 (C^8), 109.8 (C^3), 82.4 (C$^{1'}$), 69.8 (C$^{3'}$), 59.8 (C$^{4'}$ or *C*H$_2$CH$_3$), 59.7 (C$^{4'}$ or *C*H$_2$CH$_3$), 14.0 (CH$_2$*C*H$_3$) (See also Supplementary data: files a and b). Compound **3j**-1-[(2-hydroxy-ethoxy)methyl]-7-chloro-4(*1H*)quinolone-3-carboxylic acid 41%, mp 123–124 °C; IR 3384, 1708, 1608; ^{1}H NMR δ 14.78 (s, COO*H*),9.28 (s, H^2), 8.49 (t, J = 8.4 Hz, H^5), 8.47 (s, H^8), 7.83 (d, J = 8.7, H^6), 6.07 (s, H$^{1'}$), 4.74 (t, J = 5.4 Hz, O*H*), 3.70–3.73 (m, H$^{3'}$), 3.60–3.62 (m, H$^{4'}$). ^{13}C NMR δ 173.6 (C^4), 165.4 (*C*OOH), 150.7 (C^2), 140.2 (C^7); 138.4 (C^{8a}), 129.0 (C^5), 127.1 (C^{4a}), 126.4 (C^6), 118.3 (C^8), 110.6 (C^3), 70.7 (C$^{1'}$), 60.6 (C$^{3'}$), 52.3 (C$^{4'}$). (See also Supplementary data, files c and d).
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