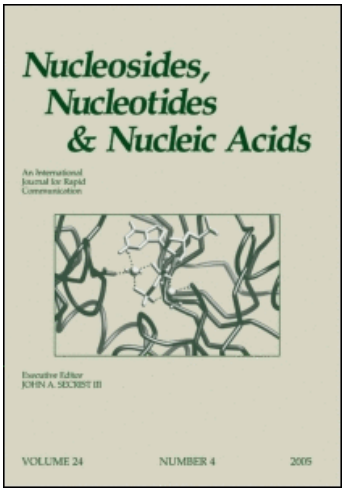

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SYNTHESIS AND ANTIVIRAL EVALUATION OF ISATIN RIBONUCLEOSIDES

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SYNTHESIS AND ANTIVIRAL EVALUATION OF ISATIN RIBONUCLEOSIDES

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ABSTRACT

A series of novel substituted isatin ribonucleosides **3b–3f** were synthesized in good yields by a TMSOTf catalysed coupling reaction between the silylated nitrogenated base (**1b–1f**) and 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (**2**). Isatin nucleoside **3a** previously reported was also prepared using this method giving high yield. From the compounds

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tested, ribonucleoside **3f** proved to be the most active one when assayed for antiviral activitiy on HSV-1 infected cells, leading to 66% of inhibition of virus yield. All the isatin derivatives tested did not inhibit HIV-1 Reverse Transcriptase (RT) activity.

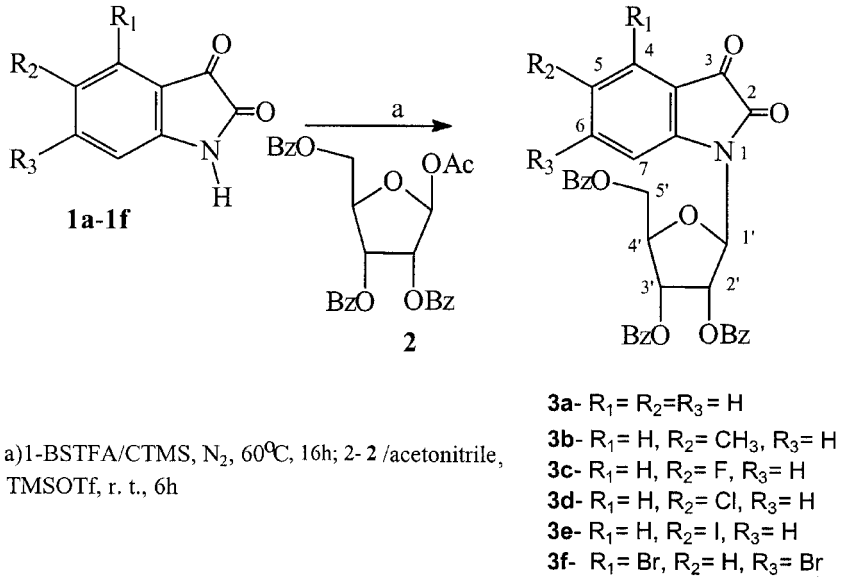
The synthesis of new ribonucleosides continues to be important for the search of therapeutically useful agents.[1] Isatin derivatives[2] have been found to possess a wide variety of pharmacological properties as follows- monoamine oxidase inhibitor,[3] antibacterial[4] and antiviral activities.[4b,4c,5] However, the literature reports few studies on the preparation of isatin nucleosides.[6–9]

As part of an ongoing program[10,11] for investigating the transformation of isatins into new derivatives with potential biological activity, this work describes the synthesis of five new isatin ribonucleosides: 5-methyl-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (**3b**), 5-fluoro-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (**3c**), 5-chloro-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (**3d**), 5-iodo-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl) indole-2,3-dione (**3e**), 4,6-dibromo-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (**3f**) as well as derivative 2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (**3a**)[6a] previously reported. These isatin ribonucleosides were screened for their in vitro antiviral properties in HSV-1 infected cells. These compounds were also tested on HIV-1 RT activity. The reaction sequence for the preparation of the target compounds is outlined in Sch. 1.

RESULTS AND DISCUSSION

Isatin derivatives used in this work (**1a**–**1f**) were prepared according to published procedures.[10,11] The method chosen for the synthesis of the corresponding ribonucleosides was the coupling between the previously silylated nitrogenated base and 1-*O*-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (**2**) using Lewis acid catalysis.[12] Heating **1** with bis(trimethylsilyl)trifluoroacetamide (BSTFA)[13] containing 1% of trimethylchlorosilane (TMSCl), at 60°C, under nitrogen atmosphere, for 16 h, followed by reaction with **2**, in the presence of trimethylsilyltrifluoroamethanesulfonate (TMSOTf)[14] as catalyst, at room temperature, for 6 h, gave high yields of the corresponding ribonucleosides **3a**–**3f** (Sch. 1) (**3a**: 90%; **3b**: 80%; **3c**: 82%; **3d**: 78%; **3e**: 78%; **3f**: 80%). The use of a mixture of hexamethyldisilazane/trimethylchlorosilane as the silylating agent and toluene as solvent led to clear solutions which failed to give any nucleoside product.

Although the de-O-benzoylation of **3a** is successfully reported in the literature,[6a] all attempts to remove the O-benzoyl groups from **3a**–**3f** using



a)1-BSTFA/CTMS, N_2, 60°C, 16h; 2- **2** /acetonitrile, TMSOTf, r. t., 6h

3a- R_1= R_2=R_3 = H
3b- R_1= H, R_2= CH_3, R_3= H
3c- R_1= H, R_2= F, R_3= H
3d- R_1= H, R_2= Cl, R_3= H
3e- R_1= H, R_2= I, R_3= H
3f- R_1= Br, R_2= H, R_3= Br

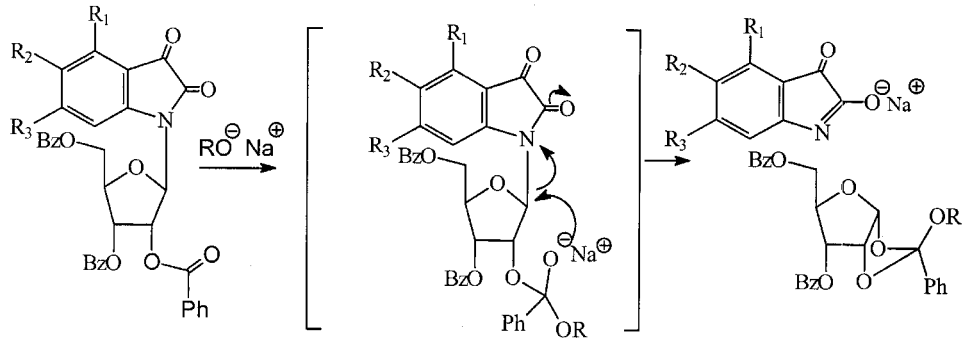
Scheme 1. Synthetic route used for the preparation of isatin nucleosides **3a**–**3f**.

different basic or acidic reagents such as methanol/sodium methoxide solution, methanol/sodium bicarbonate solution and Dowex H^+/H_2O, under different reaction conditions of time and temperature, led to nucleoside decomposition products, probably as a result of the initial fragmentation of N-glycosyl bond. This cleavage, in basic conditions, may be rationalized by the attack of the alcoxide ion on the most reactive benzoyl group (C-2), followed by a fast β-attack of the oxygen anion from the ortho-ester to the anomeric center, eliminating the isatin moiety, which is a good leaving group due to the vicinal carbonyl, as outlined in Sch. 2. Thus, it seems that this cleavage reverses the glycosyl bond by the same kind of mechanism of the regiocontrolled and stereospecific glycoylation of isatin derivatives with 1-O-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**). This cleavage was also observed in FAB mass spectra.

The structures of the nucleosides were confirmed by IR, UV, ^1H and ^{13}C NMR experiments [^1H (Table 1), ^{13}C (Table 2), DEPT, ^1H-^1H-COSY, HETCOR ($^1J_{CH}$ and $^nJ_{CH}$, n = 2, 3)] and by FAB-high resolution mass spectra which revealed the molecular-ion peaks $(M+H)^+$as well as the ion m/z 445 attributed to the riboside fragment resulting from C-N cleavage of glicosyl bond.

^1H and ^{13}C spectra of **3a**–**3f** showed very similar chemical shifts for the riboside moiety in each case (Tables 1 and 2).

The observation of nuclear Overhauser effect (n.O.e.) for the H4′ signals while the anomeric proton was being irradiated (**3a**: 3.09%; **3b**: 2.46%; **3c**: 3.72%; **3d**: 3.04%; **3e**: 3.88%; **3f**: 2.97%) confirmed the β-configuration of all nucleosides.[15] The point of attachment of the sugar to the bases assigned to the



R= H or Me

Scheme 2. Cleavage of the N-glycosyl bond of **3a**–**3f** under basic conditions.

nitrogen atom from the isatin nucleus was based upon carbon-13 NMR chemical shifts for C2 and C3. These were compared with data for N-alkylated isatins in the literature.[11] Additional proof for N1-ribosylation was also visible through the nuclear Overhauser effect in the spectra of the compounds where the irradiation of the anomeric hydrogen, H1′, resulted in an *observed* n.O.e. on H7 (**3a**: 5.45%; **3b**: 3.28%; **3c**: 6.11%; **3d**: 6.89%; **3e**: 7.11%; **3f**: 5.25%).

BIOLOGICAL STUDIES

HSV-1 infected cells were incubated with the isatins and isatin ribonucleosides at a concentration of 50 μM for 72 h. The antiviral activity was determined according to the computation of the 50 percent endpoint method described by Reed and Muench.[5] Among the heterocycles, **1c** and **1f** were found to reduce virus yield significantly. Ribonucleosides **3a**, **3b** and **3f** proved to be more active than the corresponding isatin heterocycles, while the heterocycle **1c** was more active than the corresponding ribonucleoside. Thus, these five compounds were considered to be the most efficient derivatives, leading to statistically significant inhibition of virus yield (Table 3). The most efficient isatin derivatives were still less efficient than the potent anti HSV-1 compound, acyclovir, which reduced virus yield nearly 100% at the same concentration. Sincitium formation was exclusively observed in infected cells incubated with ribonucleoside **3c**. Compound **3d** was toxic to VERO cells and therefore was not tested.

Further investigation on the antiviral activities of the heterocycles **1a**–**1f** and the ribonucleosides **3a**–**3f** was assessed by measuring [3H]dTTP incorporation in a poly(rA).oligo(dT) template primer by the activity of the

Table 1. ^{1}H (300.00 MHz) NMR Spectral Data for **3a–3f** [($CDCl_3$/TMS), J (Hz)]

^{1}H	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
4	7.66–7.58 (m)	7.43–7.33 (m)	7.30 (dd, 6.6, 2.7)	7.65–7.48 (m)	7.88 (d, 1.8)	–
5	7.13–7.06 (m)	–				7.48–7.35 (m)
6	7.13–7.06 (m)	6.90 (dd, 8.1, 1.2)	6.75 (dt, 8.8, 2.7)	7.01 (dd, 8.7, 2.1)	7.33 (dd, 8.4, 1.8)	–
7	7.24–7.21 (m)	7.10 (d, 8.1)	7.21 (dd, 3.6, 9.0)	7.18 (d, 8.7)	7.01 (d, 8.4)	7.48–7.35 (m)
1′	6.25 (d, 6.3)	6.23 (d, 6.0)	6.23 (d, 6.0)	6.19 (d, 6.3)	6.18 (d, 6.0)	6.09 (d, 6.0)
2′	6.13 (t, 6.3)	6.12 (t, 6.0)	6.10 (t, 6.3)	6.10 (t, 6.1)	6.1.0 (t, 6.3)	6.20 (t, 6.0)
3′	6.05 (dd, 6.3, 4.5)	6.04 (dd, 6.6, 4.8)	6.04 (dd, 6.3, 4.5)	6.03 (dd, 6.3, 4.5)	6.02 (dd, 6.6, 4.5)	6.07–6.03 (m)
4′	4.77–4.68 (m)	4.73–4.66 (m)	4.72–4.67 (m)	4.72–4.66 (m)	4.70–4.65 (m)	4.75–4.66 (m)
5′a	4.96–4.90 (m)	4.95–4.86 (m)	4.95 (dd, 13.2, 3.3)	4.94 (dd, 13.5, 3.9)	4.94 (dd, 13.5, 3.6)	4.94 (dd, 13.5, 3.9)
5′b	4.74–4.60 (m)	4.73–4.66 (m)	4.72–4.67 (m)	4.72–4.66 (m)	4.70–4.65 (m)	4.75–4.66 (m)
2″	8.13 (dd, 8.4, 1.5)	8.13 (dd, 8.4, 1.5)	8.12 (dd, 8.4, 1.5)	8.11 (dd, 8.4, 1.2)	8.10 (dd, 8.4, 1.2)	8.08 (dd, 8.4, 1.5)
	7.97 (dd, 8.4, 1.5)	7.97 (dd, 8.4, 1.5)	7.98 (dd, 8.4, 1.2)	7.98 (dd, 8.1, 1.2)	7.98 (dd, 8.4, 1.5)	7.99 (dd, 8.4, 1.2)
	7.93 (dd, 8.7, 1.5)	7.92 (dd, 8.4, 1.5)	7.92 (dd, 8.4, 1.5)	7.91 (dd, 8.4, 1.2)	7.91 (dd, 8.1, 1.2)	7.93 (dd, 8.4, 1.2)
3″	7.57– 7.46 (m)	7.57– 7.47 (m)	7.68–7.49 (m)	7.65–7.48 (m)	7.69–7.48 (m)	7.48–7.35 (m)
	7.41–7.33 (m)	7.43–7.33 (m)	7.43–7.30 (m)	7.30–7.33 (m)	7.43–7.31 (m)	
4″	7.66– 7.58 (m)	7.66–7.58 (m)	7.68–7.49 (m)	7.65–7.48 (m)	7.69–7.48 (m)	7.62–7.53 (m)
	7.57–7.46(m)	7.57– 7.47 (m)				
$\underline{C}H_3$	–	2.25 (s)	–	–	–	–

Table 2. ^{13}C (75.0 MHz) NMR Spectral Data for **3a–3f** [($CDCl_3$/TMS)]

^{13}C	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
2	157.6	157.8	157.6	157.0	156.6	156.4
3	181.5	181.8	180.93	180.6	180.2	179.9
3a	117.7	117.8	118.6 (d, 7.5)	118.7	119.3	115.4
4	125.6	125.8	112.3 (d, 24.0)	125.3	134.0	122.34
5	124.2	134.1	159.3 (d, 246.0)	130.1	86.9	131.5
6	138.2	138.7	124.6 (d, 23.5)	137.5	146.2	133.3
7	112.2	112.0	113.8 (d, 7)	113.6	114.3	114.4
7a	148.3	146.1	144.4	146.6	147.7	150.6
1′	84.3	84.2	84.4	84.5	84.7	84.8
2′	70.3 or 70.2	70.3 or 70.2	70.3 or 70.2	70.3	70.3	71.0
3′	70.3 or 70.2	70.3 or 70.2	70.3 or 70.2	70.3	70.3	70.6
4′	79.9	79.8	80.0	80.0	84.5	80.2
5′	63.3	63.4	63.2	63.2	63.1	63.4
1″	129.2, 128.2	129.2, 128.4, 128.1	129.2, 128.5, 128.1	129.2, 128.1	129.2, 128.1	129.2, 128.5
2″	129.7, 129.6, 129.5	129.7, 129.6, 129.5	129.7, 129.6, 129.5	129.7, 129.6, 129.5	129.7, 129.6, 129.5	129.73, 129.7, 129.6
3″	128.5, 128.4, 128.3	128.5, 128.3	128.6, 128.4, 128.3	128.6, 128.5, 128.4	128.6, 128.4	128.6, 128.4
4″	133.6, 133.5, 133.3	133.6, 133.5, 133.3	133.7, 133.6, 133.5	133.7, 133.6, 133.5	133.7, 133.6, 133.5	133.7, 133.6, 133.4
$\underline{C}H_3$		20.4	–	–		
C=O	165.8, 165.2, 165.1	165.8, 165.2, 165.1	165.8, 165.2, 165.1	165.8, 165.2, 165.1	165.8, 165.2, 165.1	166.0, 165.2, 165.1

Table 3. Antiviral Activity of Isatins **1a**–**1f** and Isatin Ribonucleosides **3a**–**3f** (50 μM) on HSV-1 Yield

Compounds	Herpes Simplex Type 1 Virus (% of Inhibition of Virus Yield)
1a	0
1b	− 16
1c	*60**
1d	28
1e	28
1f	*46**
3a	*61**
3b	*46**
3c	15
3d	N.D.
3e	37
3f	*66**

N.D. = not determined.
Vero cells (10^6 cells/well) were infected with HSV-1 at 1PFU/cell for 2 h. At zero time post-infection, virus inocullum was replaced by culture medium containing or not isatin derivatives. After 72 h post-infection, virus yield of each sample was determined by 50% tissue culture infective dose ($TCID_{50}$/mL). The data are mean values from one of the representative experiments.
*P values < 0.05 for comparing drug-treated and non-treated cells.

recombinant HIV-1 reverse transcriptase (HIV-1 RT), as already described.[17] Our results showed that none of the isatin derivatives reduced the RNA-dependent DNA polymerase activity of the recombinant HIV-1 RT.

In conclusion, the modest but significant anti-HSV-1 activities of the isatin derivatives described here suggests that these compounds may be selectively targeted to HSV-1 replication, since they were not cytotoxic to VERO cells at the concentration tested.

EXPERIMENTAL

General Procedures

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 1420 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Ultraviolet (UV) spectra were obtained on a Schimadzu spectrophotometer; λ are in nm and ε in mol^{-1} cm^{-1}. Mass spectra analysis

(FABHRMS) were recorded in a 3-nitrobenzylalcohol matrix in the positive ion mode on a VG ZAB-E mass spectrometer, by Department of Chemistry and Biochemistry, University of Oklahoma. 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 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 Procedure for the Preparation of 2,3-Dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3a), 5-Methyl-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3b), 5-Fluoro-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3c), 5-Chloro-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3d), 5-Iodo-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3e) and 4,6-Dibromo-2,3-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)indole-2,3-dione (3f). A mixture of isatin **1a**–**1f** (2.0 mmol) and bis(trimethylsilyl)trifluoroacetamide (BSTFA, 7.46 mmol) containing 1% of trimethylchlorosilane, in acetonitrile (4 mL) was stirred at 60°C, under nitrogen atmosphere, for 16 h. The reaction mixture was allowed to cool to room temperature and a solution of 1-*O*-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (2.1 mmol) in 15 mL of acetonitrile was added. This procedure was followed by the dropwise addition of a solution of TMSOTf (2.1 mmol) in 10 mL of acetonitrile. After stirring for 6 h. the resulting mixture was poured into ice-cold water (20 g). Afterwards, it was neutralized with a saturated aqueous solution of sodium bicarbonate and extracted with ethyl acetate (3 × 30 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane:methylenechloride gradient (up to 40%) as the eluant, to give:

3a: as orange crystals (1.08 g, 90%), m.p. 73°C; UV λ_{max} ($CHCl_3$) 411 (ε 426), 244 (ε 35,449); IR 1730 (C=O), 1260 (C-O); FABHRMS m/z calcd for $C_{34}H_{26}NO_9$ $(M+H)^+$ 592.1619, found 592.1607; ^{1}H NMR (300.00 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (75.0 MHz, $CDCl_3$) see Table 2.

3b: as orange crystals (0.97 g, 80%), m.p. 78°C; UV λ_{max} ($CHCl_3$) 424 (ε 406), 244 (ε 17,939); IR 1730 (C=O), 1260 (C-O); FABHRMS m/z calcd for $C_{35}H_{28}NO_9$ $(M+H)^+$ 606.1715, found 606.1171; ^{1}H NMR (300.00 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (75.0 MHz, $CDCl_3$) see Table 2.

3c: as orange crystals (1.00 g, 82%), m.p. 86°C; UV λ_{max} ($CHCl_3$) 411 (ε 633), 245 (ε 25,443); IR 1725 (C=O), 1260 (C-O); FABHRMS m/z calcd

for $C_{34}H_{25}NO_9F$ $(M+H)^+$ 610.1525, found 610.1513; ^1H NMR (300.00 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$) see Table 2.

3d: as orange crystals (0.98 g, 78%), m.p. 86°C; UV λ_{max} ($CHCl_3$) 419 (ε 609), 245 (ε 25,749); IR 1730 (C=O), 1260 (C-O); FABHRMS m/z calcd for $C_{34}H_{25}NO_9{}^{35}Cl$ $(M+H)^+$ 626.5229; found 626.1217; ^1H NMR (300.00 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (75.0 MHz, $CDCl_3$) see Table 2.

3e: as orange crystals (1.10 g, 78%), m.p. 83°C; UV λ_{max} ($CHCl_3$) 424 (ε 989), 243 (ε 22,198); IR 1730 (C=O), 1260 (C-O); FABHRMS m/z calcd for $C_{34}H_{25}NO_9I$ $(M+H)^+$ 718.0585, found 718.0574; ^1H-NMR ($CDCl_3$) see Table 1; ^{13}C-NMR ($CDCl_3$) see Table 2.

3f: as yellow crystals (1.20 g, 80%), m.p. 223°C; UV λ_{max} ($CHCl_3$) 406 (ε 922), 243 (ε 19,324); IR 1730 (C=O), 1260 (C-O); FABHRMS m/z calcd for $C_{34}H_{24}NO_9{}^{81}Br_2$ $(M+H)^+$ 751.9790, found 751.9788; ^1H NMR (300.00 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (75.0 MHz, $CDCl_3$) see Table 2.

Cells. VERO epithelial cell line derived from monkey kidney cells obtained from the American tissue culture Collection (Rockville, MD) were grown in monolayer cultures in Dulbecco's modified Eagle's medium (Gibco laboratories) containing 2% heated-inactivated fetal bovine serum (FBS; Pigue Farm), 8% calf serum (CS; Pan-American Center of aftose fever), 2.25% sodium bicarbonate, 500 U/mL penicilin, 100 μg/mL streptomycin, 50 μg/mL gentamycin, 2.5 μg/mL fungison. Cell cultures were incubated at 37°C in humidified air containing 5% CO_2. For passaging, confluent monolayers (6.5×10^5 cells) were washed with phosphate-buffered saline solution (NaCl, 0.8 g%, KCl, 0.02 g%, Na_2HPO_4, 0.15 g%, NaH_2PO_4, 0.02 g%, EDTA, 1 mM PBS pH 7.2), and after a short trypsinisation (Trypsin at 0.25 g%) the cells were suspended in the culture medium.

Virus. HSV-1 were kindly provided by Dr. Marcia Wigg (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil), propagated in Hep-2-cells and stored at −70°C until use.

Antiviral Activity. Antiviral activity on HSV-1 virus infection was assessed by virus yield assay. Briefly, sub-confluent VERO cells, grown in 96-well plates (10^6 cells/well), were infected with HSV-1 at 1 PFU (plaque forming unity)/cell for 120 min at 37°C in 100 μL of DMEM. After this period, virus inocullum was replaced by culture medium, containing isatin derivatives or not. We defined this point as the zero time post-infection. After 72 h post-infection, culture medium was harvested and virus titer of each sample was quantified in terms of the 50% tissue culture infective dose ($TCID_{50}$/mL) by endpoint dilution, according to Reed and Muench.[16]

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