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Original article

Synthesis and antiviral activity of β-carboline derivatives bearing a substituted carbohydrazide at C-3 against poliovirus and herpes simplex virus (HSV-1)

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ABSTRACT

Several novel 1,3-disubstituted β-carboline derivatives bearing a substituted carbohydrazide group at C-3 were synthesized and evaluated for their antiviral activity against vaccinal poliovirus (VP) and herpes simplex virus type 1 (HSV-1). The cytotoxicity and selectivity index of the active compounds were also evaluated. Among the synthesized derivatives, compounds **10** and **11** displayed potent activity against both vaccinal poliovirus and HSV-1 virus. Compound **10** presented the highest selectivity index (SI = 2446.8) against HSV-1 virus and low cytotoxicity ($CC_{50} = 1150.0 \pm 67.3$ μM). The virus yield inhibition assay showed that compound **10** was able to inhibit HSV-1 plaque formation before and during the virus adsorption. The characteristic small plaque pattern observed in compound-treated cells suggested that compound **10** inhibited viral dissemination to neighboring cells. A computational study for prediction of ADME properties of the novel synthesized β-carbolines derivatives was performed by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors, using Lipinski's rule.

1. Introduction

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are human herpes viruses belonging to family Herpesviridae [1]. These types of HSV are responsible for mucocutaneous infections, mainly in immunocompromised patients. HSV-1 provokes orofacial lesions, while HSV-2 causes mucocutaneous genital infections [2]. Antiviral research on HSV preliminarily focuses on compounds capable of targeting the viral polymerase. Acyclovir, a nucleoside inhibitor of DNA polymerase, the first selective antiviral agent introduced, still is the drug commonly employed in the treatment of HSV infection [3]. However, the widespread use of Acyclovir has led to the development of viral resistance against this drug [4]. Therefore, the search for new drugs against Acyclovir-resistant HSV viruses is highly necessary.

Poliovirus (PV) is a member of the genus *Enterovirus* belonging to Picornaviridae family. This non-enveloped virus with a single stranded RNA genome is the etiological agent of poliomyelitis, a disease under control in most countries [5]. However, the members of this genus cause a wide array of illnesses, such as, meningitis, myocarditis, encephalitis, and respiratory diseases. Although well studied, poliovirus remains one of most appropriate model for the study of viral replication.

Recent studies have pointed to β-carboline alkaloids as a new class of antiviral agents. Harman and some of its derivatives and matairesinol, a compound isolated from *Symplocos setchuenensis*, were found to inhibit the replication of HIV in H9 lymphocyte cells [6]. Flazin, a β-carboline alkaloid isolated of *Suillus granulatus* was found to possess anti-HIV activity [7]. A structure–activity study on 46 flazin synthesized analogues showed a potent anti-HIV activity for flazinamide, which was considered a promising anti-HIV agent [8]. Synthetic β-carboline derivatives bearing a guanidinium group-terminated side chain at C-3 exhibited anti-HIV activity in MT4 cells, inhibiting HIV replication by interfering with TAT–TAR interaction [9].

The potentialities of β-carboline alkaloids as antiviral agents and the importance of the search for new anti-HSV-1 drugs have led us to study this class of compounds. Studies on structure–activity relationship have demonstrated the influence of the substituents in positions-1, -3, and -9 of the β-carboline skeleton for a variety of

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synthetic β-carboline derivatives [10–19]. Furthermore, antiviral activity has been reported for several compounds possessing the carbohydrazide moiety [20–23].

Based on these considerations, in this work, we synthesized a series of novel β-carboline derivatives bearing a substituted carbohydrazide group at C-3 and a phenyl-substituted group at C-1. All derivatives were evaluated for their *in vitro* activity against herpes simplex virus type 1 (HSV-1) and vaccinal poliovirus (VP). The cytotoxicity and selectivity index of the active compounds were determined. Studies to establish the step of the viral cycle affected were also performed. A computational study for prediction of ADME properties of the novel synthesized β-carbolines derivatives was carried out by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors, using Lipinski's rule.

2. Results and discussion

2.1. Chemistry

The synthetic route for the preparation of β-carboline-3-carbohydrazides **5**–**23** is presented in Scheme 1. Methyl tetrahydro-β-carboline-3-carboxylates **2a**–**e** were prepared through Pictet–Spengler condensation of L-tryptophan **1** with 4-hydroxybenzaldehyde (**a**), benzaldehyde (**b**), 4-methoxybenzaldehyde (**c**), 3-nitrobenzaldehyde (**d**) and 4-nitrobenzaldehyde (**e**), in acid media, and subsequent esterification of the corresponding carboxylic acids with methanol and sulfuric acid [13,24]. Oxidation of methyl 1,2,3,4-tetrahydro β-carboline-3-carboxilates **2a**–**e** with sulfur under xylene reflux [15,25] furnished methyl β-carboline-3-carboxylates **3a**–**e**. Conversion of **3a**–**e** to 1-(substituted-phenyl)-β-carboline-3-carbohydrazides **4a**–**e** was carried out by reaction with hydrazine hydrate in ethanol under reflux according to the procedures described in literature for similar compounds [15]. Condensation of **4a**–**e** with aromatic aldehydes 4-methoxybenzaldehyde, 4-dimethylaminobenzaldehyde, benzaldehyde, 4-nitrobenzaldehyde, and 2-chlorobenzaldehyde under reflux in ethanol yielded the carbohydrazides **5**–**23**.

All novel compounds were characterized by their spectral data (IR, EIMS, ^{1}H and ^{13}C NMR). The ^{1}H NMR spectra of carbohydrazides **5**–**23** showed signals at δ_H 8.40 integrating for one proton, and at δ_H 6.70–9.00, corresponding to the imine and aromatic hydrogens, respectively, of the (substituted-benzylidene)carbohydrazide group. The presence of this group was confirmed by the signals at δ_C 147–150 (C=N), 158–162 (C=0), and δ 110–135 (aromatic carbons of R^2 group) in the ^{13}C NMR spectra.

2.2. Antiviral activity

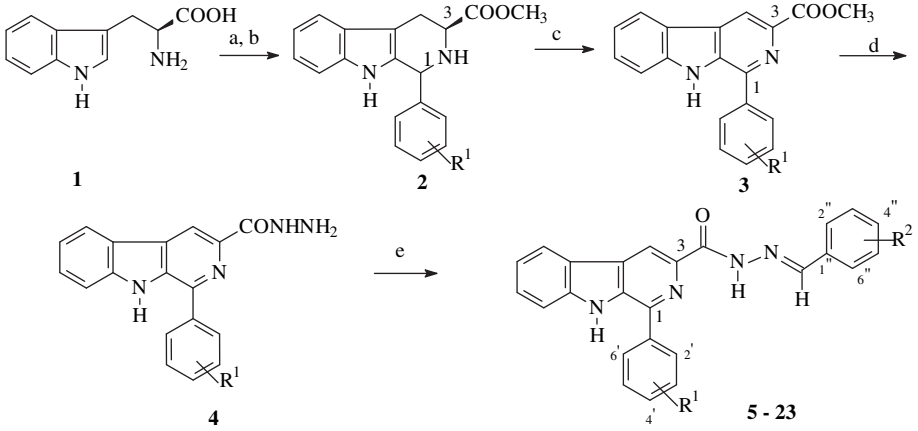
The novel β-carboline-3-carbohydrazides derivatives **5**–**23** were evaluated for their activity towards HSV-1 and vaccinal poliovirus (VP). Compounds with $EC_{50} > 100$ μM were considered inactive. The cytotoxicity to Vero cells and the selectivity index for the active derivatives were also determined.

The antiviral assay results (Table 1) showed that five (**6**, **7**, **10**–**12**) of all tested compounds were active towards poliovirus, whereas six (**5**, **8**–**11** and **20**) displayed potent-to-weak activity against HSV-1. The β-carboline-3-carbohydrazides **10** and **11**, which have a phenyl group at C-1, and a 4-methoxybenzylidene-carbohydrazide and 4-*N*,*N*-dimethylaminobenzylidene-carbohydrazide groups at C-3, respectively, displayed potent activity against both poliovirus and HSV-1 with low cytotoxicity and high selectivity index.

Comparison of the EC_{50} data of all tested compounds showed that the electronic nature of the R^1 and R^2 substituents on the phenyl portions affects the antiviral activity to a significant extent. The presence of an electron-withdrawing substituent, such as nitro or chlorine in the phenyl group attached to C-1 of the β-carboline skeleton or to the carbohydrazide moiety, resulted in inactive (**13**, **14**, **18**, **19** and **21**–**23**) or weakly active (**8**, **9**, and **20**) compounds. Specifically for the most active compound **10**, the substitution of the 4-methoxybenzylidene-carbohydrazide group for 3-nitrobenzylidene-carbohydrazide or 2-chlorobenzylidene-carbohydrazide groups led, respectively, to the inactive compounds **13** and **14**.

Concerning the influence of the electron-donating groups on the phenyl substituent, it was observed that the activity depends on both nature of the substituent and its position, if at C-1 of the β-carboline or on the carbohydrazide moiety. The substitution of the 1-(4-hydroxyphenyl) groups of the anti-PV active compounds **6** and **7** for 1-(4-methoxyphenyl) group resulted in inactive compounds **16** and **17**. Comparison of EC_{50} values of compounds **12** and **10** showed that the presence of a 4-methoxy substituent (R^2) at the phenyl of carbohydrazide moiety increase the activity of **10**. However, the change of the 4-methoxyphenyl group from the carbohydrazide moiety (in compound **10**) to the C-1 of β-carboline nucleus resulted in the inactive compound **17**.

To establish the step in which compounds **6** and **10** affects the VP and HSV-1 viral cycle, respectively, the virus yield inhibition assay was performed before, during, and after virus infection. The experiments were performed on Vero cell monolayers in 24-well plates. The results showed that β-carboline derivative **6** have no effect on poliovirus neither on adsorption (before) nor during virus



Scheme 1. Reagents and conditions: (a) acetic acid, R^1CHO, reflux, 2 h, and adjusted to pH = 5 with NH_4OH; 80–90%. (b) CH_3OH, H_2SO_4, reflux, 48 h; 82–87%. (c) S, xylene, reflux, 48 h to 4° C, 3 h; 70–73%. (d) $NH_2NH_2.H_2O$, EtOH, reflux, 48 h; 72–76%. (e) R^2CHO, EtOH, H_2SO_4 (cat), reflux, 36 h; 60–78%.

Table 1
HSV-1 and poliovirus antiviral activity, cytotoxicity and selectivity index (SI) data for β-carboline-3-carbohydrazide derivatives **5–23**.

Comp	R^1	R^2	CC_{50}[a] (μM)	PV		HSV-1	
				EC_{50}[b] (μM)	SI[c]	EC_{50}[b] (μM)	SI[c]
5	4-OH	4-OCH_3	802.7 ± 100.5	>100	nd	22.9 ± 7.3	35.0
6	4-OH	4-$N(CH_3)_2$	900.2 ± 94.5	2.67 ± 1.24	337.2	>100	nd
7	4-OH	H	1238.9 ± 487.6	1.03 ± 0.17	1202.8	>100	nd
8	4-OH	4-NO_2	199.5 ± 80.2	>100	nd	86.5 ± 5.4	2.3
9	4-OH	2-Cl	29.8 ± 10.1	>100	nd	41.2 ± 23.7	0.7
10	H	4-OCH_3	1150.0 ± 67.3	0.87 ± 0.5	1321.8	0.47 ± 0.3	2446.8
11	H	4-$N(CH_3)_2$	1164.6 ± 208.1	0.87 ± 0.04	1338.6	1.85 ± 0.63	629.5
12	H	H	776.9 ± 1.7	1.95 ± 1.7	398.4	>100	nd
13	H	4-NO_2	nd	>100	nd	>100	nd
14	H	2-Cl	nd	>100	nd	>100	nd
15	4-OCH_3	4-OCH_3	nd	>100	nd	>100	nd
16	4-OCH_3	4-$N(CH_3)_2$	nd	>100	nd	>100	nd
17	4-OCH_3	H	nd	>100	nd	>100	nd
18	4-OCH_3	4-NO_2	nd	>100	nd	>100	nd
19	3-NO_2	4-OCH_3	nd	>100	nd	>100	nd
20	3-NO_2	4-$N(CH_3)_2$	1049.6 ± 100.5	>100	nd	73.2 ± 7.6	14.3
21	4-NO_2	4-OCH_3	nd	>100	nd	>100	nd
22	4-NO_2	4-$N(CH_3)_2$	nd	>100	nd	>100	nd
23	4-NO_2	H	nd	>100	nd	>100	nd

nd = not determined.
[a] Concentration at which 50% cytotoxicity is observed.
[b] Concentration at which 50% efficacy in antiviral assay is observed.
[c] Selectivity index (CC_{50}/EC_{50}).

infection. Virucidal activity was also not detected (Table 2). The interference was observed at the replication viral phase (after infection) and was dose-dependent (Fig. 1A). In this phase, the viral RNA is translated in a single highly autocatalytic polyprotein, whose proteolytic cleavage products serve as capsid precursors and replication proteins. These proteins are used to viral RNA replication [26].

To determine whether compound **10** blocks HSV replication or not, we investigated its action after virus infection. The effect of compound **10** on HSV-1 was also assayed by incubating the virus with the compound for 1 h before its addition to the Vero cells. Interestingly the compound did not inhibit virus replication after infection, but it was able to interfere in the early stage of the virus infection at EC_{50} = 113 μM (Fig. 1B).

2.3. Computational study

A computational study of the synthesized β-carboline-3-carbohydrazides **5–23** was performed by determination of Lipinski's rule and topological polar surface area (TPSA). Calculations were performed using *Molinspiration online property calculation toolkit* (http://www.molinspiration.com). Table 3 shows the Lipinski parameters, the calculated percent absorption (% ABS), and the topological polar surface area (TPSA) of synthesized compounds **5–23**. The percent absorption was estimated using equation: % ABS = 109 − 0.345 × TPSA, according to Zhao et al. [27].

In vivo absorption of the synthesized β-carboline-3-carbohydrazides was tentatively assessed by means of theoretical calculations following Lipinski's rule of five [28], which establishes that the absorption or permeation of an orally administered compound is more likely to be good if the drug satisfies the following criteria: a) hydrogen bond donors ≤5 (OH and NH groups); b) hydrogen bond acceptors ≤10 (N and O atoms); c) molecular weight <500; d) calculated log P < 5. Compounds violating more than one of these rules may present bioavailability problems. Our results revealed that the lipophilicity of all β-carboline-3-carbohydrazides is larger than 5.0; however, the molecular weight (390.4 > MW < 480.4), hydrogen bond acceptors (n-ON = 5–9) and donors (n-OHNH = 2–3) fulfill Lipinski's rule. These data may suggest the potential of these derivatives as new antiviral agents.

Accordingly, the calculated percent absorption of compounds **5–23** ranged between 53.2 and 84.8%. The compounds with the 4-nitrophenyl group at C-1 of the β-carboline skeleton or attached to the carbohydrazide moiety showed high TPSA values, suggesting their poor oral bioavailability and absorption. However, the most potent compounds **6**, **7**, and **10–12** demonstrated better TPSA than ACV (Acyclovir) (%ABS = 67.9%), presenting % ABS between 76.7 and 84.8%.

In order to identify the compounds with potential drug-score, the most active β-carboline-3-carbohydrazide derivatives **6**, **7**, and **10–12** were submitted to an *in silico* ADMET screening (available at http://www.organic-chemistry.org). There are several approaches that assess druglikeness of compounds based on topological descriptors, fingerprints of molecular drug-like structures keys, or other properties, such as *c*log *P* and molecular weight [29]. The Osiris program (www.organic-chemistry.org/prog/peo) determines the frequency of occurrence of each fragment within the collection of treated drugs and within the supposedly non-drug-like collection of Fluka compounds. Positive values (0.1–10) indicate that the molecule predominantly contains the better fragments, which are frequently present in commercial drugs. The drug-score combines druglikeness, *c*log *P*, log *S*, molecular weight, and toxicity risk in one handy value than may be used to judge the compound overall potential to qualify for a drug.

In this work, we used the Osiris program to calculate the fragment-based druglikeness and drug-score of the most active compounds **6**, **7**, and **10–12** (Fig. 2). The data were compared with

Table 2
Effect of time treatment on antipoliovirus activity of compound **6** (n = 1).

	% Reduction of plaque formation			
	0.825 μM	1.65 μM	3.3 μM	6.6 μM
Before	0	0	0	0
During	0	0	0	0
After	0	32.0	66.3	88.3
Virucidal	0	0	0	0

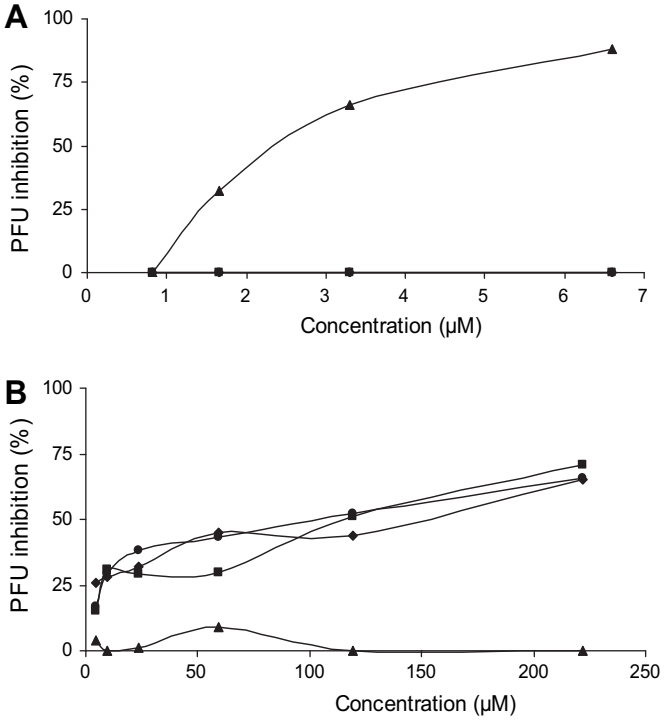


Fig. 1. Effect of the compound **6** on poliovirus (A) and **10** on HSV-1 infection (B). Compound was added before (◆), during (●), and after virus infection (▲) on Vero cell monolayers. Virucidal activity (■) was also investigated. Antiviral activity of compound **6** on poliovirus was only due its effect after virus infection, while the compound **10** showed to be active on HSV-1 by inhibiting the adsorption/penetration step or inactivating the virus particle. Data are means ± standard deviation of triplicate samples of a representative experiment. Each experiment was repeated at least three times.

those calculated for ACV. Interestingly, derivatives **6**, **7**, **10**, **11**, and **12** presented druglikeness values (from 3.307 to 4.517) better than ACV (−2.418). The drug-score values of the most active compounds were smaller than ACV, probably due to their low solubility and high lipophilicity. Compounds **6** and **11** presented high tumorigenic risk, which is a direct indication of its non-potential drug-score.

3. Conclusion

In conclusion, some β-carboline derivatives containing (substituted-benzylidene)carbohydrazide group at C-3 were prepared and identified as novel antiviral agents against HSV-1 and poliovirus at non-cytotoxic concentrations. The high selectivity indexes (SI) obtained for the active compounds suggest that the derivatives are promising antiviral drugs. The most active compounds **6**, **7**, **10**, **11** and **12** presented better druglikeness values than that of Acyclovir; however their drug-score values were smaller than ACV, probably due to their low solubility and high lipophilicity.

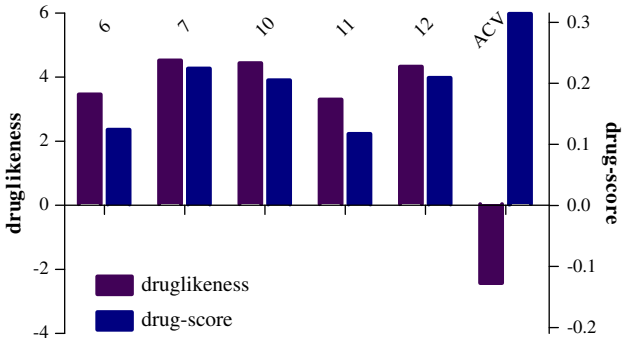


Fig. 2. Druglikeness and drug-score values of the most active compounds (**6**, **7**, **10**, **11**, **12**) and Acyclovir (ACV) using Osiris program.

Compound **10**, with a phenyl group at C-1 and a 4-methoxybenzylidene-carbohydrazide group at C-3, was the most active of the synthesized derivatives, with EC_{50} of 0.47 μM and 0.87 μM against HSV-1 and poliovirus, respectively. Our results confirmed that compound **10** exerts a dose-dependent effect during the early phases of HSV-1 infection in Vero cells. The anti-herpetic activity of this compound could be due to other mechanisms than the competition with the virus to bind to heparan sulfate (HS), the major glycosaminoglycan on the cell surface, and glycoproteins on the viral envelope [30], on the cell surface, as suggested by Marchetti et al. [31]. Further studies are required to explore the antiviral mechanism of this compound in detail.

4. Experimental protocols

4.1. General

^{1}H and ^{13}C spectra were recorded in a Varian spectrometer model Mercury plus BB at 300 MHz and 75 MHz, respectively. Mass spectra (MS) were recorded in a Thermoelectron Corporation Focus-DSQ II spectrometer. IR spectra were recorded on a BOMEM spectrometer model MB-100. For TLC, Merck precoated plates (silica gel 60 G254) were used. Silica gel 60 Merck (230–400 mesh) was used in column chromatography purification of some compounds. All reagents were purchased from commercial suppliers.

4.2. Synthesis

4.2.1. General procedure for preparation of 1-(substituted-phenyl)-N′-(substituted-benzylidene)-β-carboline-3-carbohydrazides (**5**–**24**)

A solution of derivatives **4** (1 mmol) in water (10 mL) containing two drops of concentrated sulfuric acid was refluxed for 30 min, until complete dissolution. Then, a solution of aromatic aldehyde (1 mmol) in ethanol (3 mL) was added. The resulting solution was refluxed for 48 h. The mixture was poured into cold water and neutralized with 10% aqueous sodium bicarbonate solution and the precipitate formed was filtered and dried, furnishing the title compounds **5**–**23** with 60–78% yield.

*4.2.1.1. 1-(4-Hydroxyphenyl)-N′-(4-methoxybenzylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide (**5**).* Yield: 75%; mp: 172–174 °C; IR (KBr) ν_{max} (cm^{-1}): 3442 (N–H), 1666 (C═O), 1509–1305 (C═C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (3H, s, OCH3), 6.94 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 7.34 (1H, t, $J = 7.5$ Hz, H-6), 7.58 (1H, t, $J = 7.5$ Hz, H-7), 7.65 (2H, d, $J = 7.5$ Hz, H-3′/H-5′), 7.68 (1H, d, $J = 7.5$ Hz, H-8), 7.77 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 8.13 (2H, d, $J = 8.0$ Hz, H-2′/H-6′), 8.23 (1H, d, $J = 7.5$ Hz, H-5), 8.41 (1H, s, N═CH), 8.94 (1H, s, H-4), 11.45–11.60 (s, 2H, NH); ^{13}C NMR (DMSO-d_6): δ 54.5, 112.0, 113.0, 113.2, 119.6, 120.7, 120.8, 126.0, 127.7, 128.0, 128.1, 128.3, 128.4, 129.4, 134.3, 137.0, 137.8, 140.4, 141.0, 147.2, 160.3, 160.6; MS (*m*/*z*, relative intensity %): 436.06 ($M^{+\cdot}$, 0.1), 287 (73), 260. (79), 259 (100).

*4.2.1.2. 1-(4-Hydroxyphenyl)-N′-(4-N,N-dimethylaminobenzylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide (**6**).* Yield: 78%; mp: 245–247 °C; IR (KBr) ν_{max} (cm^{-1}): 3427 (N–H), 1670 (C═O), 1520–1310 (C═C); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): δ 3.10 (6H, s, $N(CH_3)_2$),

Table 3
Predicted ADME, Lipinski parameters and molecular properties of the synthesized compounds **5**–**23** and of Acyclovir (ACV).

Comp	% ABS	TPSA[a]	Lipinski rule[a]					Log *S*[b]
			n-ON acceptors	*n*-OHNH donors	mi log *P*	MW	*n* violations	
5	74.6	99.61	7	3	5.24	436.5	1	−6.48
6	76.7	93.61	7	3	5.29	449.5	1	−6.49
7	77.8	90.37	6	3	5.18	406.4	1	−6.46
8	62.0	136.2	9	3	5.14	451.4	1	−6.92
9	77.8	90.37	6	3	5.81	440.9	1	−7.19
10	81.6	79.38	6	2	5.72	420.5	1	−6.77
11	83.7	73.38	6	2	5.77	433.5	1	−6.79
12	84.8	70.15	5	2	5.66	390.4	1	−6.75
13	69.0	116.0	8	2	5.62	435.4	1	−7.21
14	84.8	70.15	5	2	6.29	424.9	1	−7.49
15	78.4	88.61	7	2	5.78	450.5	1	−6.79
16	80.5	82.62	7	2	5.82	463.5	1	−6.81
17	81.6	79.38	6	2	5.72	420.5	1	−6.77
18	65.8	125.2	9	2	5.68	465.5	1	−7.23
19	65.8	125.2	9	2	5.65	465.5	1	−7.23
20	67.9	119.2	9	2	5.70	478.5	1	−7.25
21	65.8	125.2	9	2	5.68	465.5	1	−7.23
22	67.9	119.2	9	2	5.72	478.5	1	−7.25
23	69.0	116.0	8	2	5.62	435.4	1	−7.21
ACV	67.9	119.1	8	4	−1.61	225.2	0	−1.62

%ABS = 109 − 0.345 × TPSA.
[a] www.molinspiration.com/cgi-bin/properties.
[b] www.organic-chemistry.org/prog/peo.

6.71 (2H, d, J = 7.5 Hz, H-3′/H-5′), 7.71–7.58–7.66 (5H, m, Ar–H), 7.80 (2H, d, J = 8.0 Hz, H-2″/H-6″), 8.07 (2H, d, J = 7.5 Hz, H-2′/H-6′), 8.26 (1H, d, J = 7.8 Hz, H-5), 8.39 (1H, s, N=CH), 9.13 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 56.6, 112.4, 112.6, 113.8, 120.2, 121.4, 121.5, 128.5, 128.7, 128.8, 128.9, 130.1, 130.2, 134.8, 137.3, 138.2, 140.8, 141.6, 148.4, 150.5, 150.7, 160.7; MS (*m*/*z*, relative intensity %): 449.05 ($M^{+\cdot}$, 4.3), 287 (43), 260 (97), 259 (100).

*4.2.1.3. 1-(4-Hydroxyphenyl)-*N′*-(benzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**7**).* Yield: 83%; mp: 310–312 °C; IR (KBr) ν_{max} (cm^{-1}): 3304 (N–H), 1657 (C=O), 1534–1314 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.15 (2H, d, J = 7.8 Hz, H-3′/H-5′), 7.37 (1H, t, J = 7.8 Hz; H-6), 7.55–7.40 (3H, m, Ar–H), 7.66 (1H, d, J = 7.8 Hz, H-8), 7.84 (2H, d, J = 7.8 Hz, H-2′/H-6′), 7.95 (2H, d, J = 8.0 Hz; H-2″/H-6″), 8.27 (1H, d, J = 8.0 Hz, H-5), 8.33 (1H, s, N=CH), 8.93 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 112.1, 114.0, 116.0, 120.7, 121.9, 122.0, 127.8, 128.5, 128.7, 129.4, 129.8, 130.2, 130.5, 133.8, 135.2, 136.8, 138.2, 141.3, 148.5, 150.9, 158.1; MS (*m*/*z*, relative intensity %): 406.04 ($M^{+\cdot}$, 5.8), 303 (76), 286 (37), 260 (91), 259 (100).

*4.2.1.4. 1-(4-Hydroxyphenyl)-*N′*-(4-nitrobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**8**).* Yield: 80%; mp: 342–344 °C; IR (KBr) ν_{max} (cm^{-1}): 3380 (N–H), 1675 (C=O), 1524–1310 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.08 (2H, d, J = 7.5 Hz, H-3′/H-5′), 7.35 (1H, t, J = 7.5 Hz, H-6), 7.60 (1H, t, J = 7.5 Hz, H-7), 7.73 (1H, d, J = 7.5 Hz, H-8), 8.03 (2H, d, J = 8.0 Hz, H-3″/H-5″), 8.13 (2H, d, J = 7.5 Hz, H-2′/H-6′), 8.34 (2H, d, J = 8.0 Hz, H-2″/H-6″), 8.47 (1H, d, J = 7.5 Hz, H-5), 8.80 (1H, s, N=CH), 8.91 (1H, s, H-4), 12.06–11.86 (2H, s, NH); ^{13}C NMR (DMSO-d_6): δ 112.7, 113.5, 115.6, 120.2, 122.1, 121.2, 124.1, 127.9, 128.5, 129.5, 130.1, 130.3, 134.3, 138.4, 140.9, 141.2, 141.4, 145.5, 147.7, 158.6, 161.6; MS (*m*/*z*, relative intensity %): 451.03 ($M^{+\cdot}$, 2.5), 303 (75), 286 (29), 260 (91), 259 (100), 258 (32).

*4.2.1.5. 1-(4-Hydroxyphenyl)-*N′*-(2-chlorobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**9**).* Yield: 70%; mp: 360–362 °C; IR (KBr) ν_{max} (cm^{-1}): 3354 (N–H), 1652 (C=O), 1515–1319 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05 (2H, d, J = 7.8 Hz, H-3′/H-5′), 7.31 (1H, t, J = 7.8 Hz, H-6), 7.56–7.46 (4H, m, Ar–H), 7.60 (1H, t, J = 7.8 Hz, H-7), 7.70 (1H, d, J = 7.8 Hz, H-8), 7.95 (2H, d, J = 8.0 Hz, H-2″/H-6″), 8.00 (2H, d, J = 7.8, H-2′/H-6′), 8.45 (1H, d, J = 8.1 Hz, H-5), 8.88 (1H, s, N=CH), 9.86 (1H, s, H-4), 11.83–12.15 (2H, s, NH); ^{13}C NMR (DMSO-d_6): δ 112.7, 113.5, 115.5, 120.2, 121.2, 122.1, 127.0, 127.5, 128.2, 128.5, 129.4, 129.9, 130.4, 131.3, 132.0, 133.2, 134.2, 138.8, 141.4, 143.9, 158.9, 161.8; MS (*m*/*z*, relative intensity %): 440.01 ($M^{+\cdot}$, 1.4), 303 (90), 286 (40), 260 (97), 259 (100), 258 (33).

*4.2.1.6. 1-(Phenyl)-*N′*-(4-methoxybenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**10**).* Yield: 70%; mp: 218–220 °C; IR (KBr) ν_{max} (cm^{-1}): 3365 (NH), 1683 (C=O), 1509–1303 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.86 (3H, s, OCH_3), 6.97 (2H, d, J = 8.7 Hz, H-3″/H-5″), 7.37 (1H, td, J = 7.8; 2.5 Hz, H-6), 7.55–7.61 (3H, m, Ar–H), 7.64 (1H, d, J = 7.8 Hz, H-8), 7.69 (1H, td, J = 7.8 and 2.5 Hz, H-7), 7.81 (2H, d, J = 8.7 Hz, H-2″/H-6″), 8.04 (2H, d, J = 7.0 Hz, H-2′/H-6′), N=CH: 8.23 (1H, s, N=CH), 8.27 (1H, d, J = 8.1 Hz, H-5), 8.97 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 55.5, 112.1, 114.3, 114.5, 121.1, 122.1, 122.3, 126.9, 128.5, 129.1, 129.3, 129.4, 129.6, 130.3, 130.6, 135.2, 137.9, 139.1, 141.0, 148.2, 161.4, 161.6; MS (*m*/*z*, relative intensity %): 420.08 ($M^{+\cdot}$, 6.1), 287 (78), 270 (33), 244 (95), 243 (100), 242 (47).

*4.2.1.7. 1-(Phenyl)-*N′*-(4-*N,N*-dimethylaminobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**11**).* Yield: 75%; mp: 230–232 °C; IR (KBr) ν_{max} (cm^{-1}): 3376 (NH), 1664 (C=O), 1519–1303 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.04 (6H, s, $N(CH_3)_2$), 6.35 (2H, d, J = 6.0 Hz, H-3″/H-5″), 7.34 (1H, td, J = 7.0 and 2.0 Hz, H-6), 7.55 (1H, td, J = 7.0 and 2.0 Hz, H-7), 7.57–7.64 (3H, m, Ar–H), 7.59 (2H, d, J = 6.0 Hz, H-2″/H-6″), 7.66 (1H, d, J = 7.0 Hz, H-8), 8.03 (2H, d, J = 7.2 Hz, H-2′/H-6′), 8.13 (1H, s, N=CH), 8.24 (1H, d, J = 7.8 Hz, H-5), 8.93 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 40.1, 111.8, 112.2, 114.2, 120.8, 121.3, 122.0, 128.5, 128.8, 129.2, 129.3, 130.1, 130.5, 135.1, 137.9, 138.7, 141.2, 141.4, 149.9, 152.0, 161.1; MS (*m*/*z*, relative intensity %): 433.09 ($M^{+\cdot}$, 25.5), 287 (42), 270 (30), 244 (100), 243 (95), 242 (46).

*4.2.1.8. 1-(Phenyl)-*N′*-(benzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**12**).* Yield: 74%; mp: 290–292 °C; IR (KBr) ν_{max} (cm^{-1}): 3327 (NH), 1665 (C=O), 1518–1309 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.38 (1H, td, J = 7.0 and 2.5 Hz, H-7), 7.40–7.70 (7H, m, Ar–H), 7.61 (1H, d, J = 7.0 Hz, H-8), 7.86 (2H, d, J = 7.0 Hz,

H-2″/H-6″), 8.04 (2H, d, $J = 7.0$ Hz, H-2′/H-6′), 8.26 (1H, d, $J = 7.8$ Hz, H-5), 8.28 (1H, s, N=CH), 8.97 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 112.3, 114.5, 120.8, 121.8, 121.9, 127.7, 128.4, 128.7, 128.9, 129.1, 129.2, 130.4, 130.2, 130.5, 133.8, 135.3, 138.2, 141.4, 141.7, 148.5, 162.0; MS (*m*/*z*, relative intensity %): 390.08 ($M^{+\cdot}$, 4.2), 287 (72), 270 (32), 244 (88), 243 (100), 242 (46).

*4.2.1.9. 1-(Phenyl)-*N′*-[1-(4-nitrobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**13**).* Yield: 78%; mp: 178–180 °C; IR (KBr) ν_{max} (cm^{-1}): 3422 (NH), 1665 (C=O), 1517–1339 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.33 (1H, t, $J = 7.8$ Hz, H-6), 7.40–7.60 (3H, m, Ar–H), 7.62 (1H, t, $J = 7.8$ Hz, H-7), 7.68 (1H, d, $J = 7.8$ Hz, H-8), 8.00 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 8.20 (2H, d, $J = 7.5$ Hz, H-2′/H-6′), H-3″/H-5″, 8.31 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 8.48 (1H, d, $J = 7.8$ Hz, H-5), 8.78 (1H, s, N=CH), 8.98 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 112.7, 114.4, 120.4, 121.2, 122.2, 124.2, 127.9, 129.0, 129.2, 129.3, 129.4, 134.6, 137.3, 138.6, 140.9, 141.1, 141.6, 145.0, 147.7, 161.5; MS (*m*/*z*, relative intensity %): 435.05 ($M^{+\cdot}$, 3.7), 287 (63), 244 (85), 243 (100), 242 (43).

*4.2.1.10. 1-(Phenyl)-*N′*-(2-chlorobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**14**).* Yield: 72%; mp: 204–206 °C; IR (KBr) ν_{max} (cm^{-1}): 3446 (NH), 1671 (C=O), 1519–1322 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.30–7.55 (6H, m, Ar–H), 7.62 (1H, d, $J = 7.8$ Hz, H-8), 7.66 (2H, d, $J = 7.5$ Hz, H-3′/H-5′), 7.70 (1H, m, H-7), 8.03 (2H, d, $J = 8.1$ Hz, H-2′/H-6′), 8.23 (1H, d, $J = 7.8$ Hz, H-5), 8.29 (1H, dd, $J = 7.0$ and 3.0 Hz, H-3″), 8.96 (1H, s, H-4), 8.78 (1H, s, N=CH); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 112.3, 114.6, 120.8, 121.8, 121.9, 127.2, 127.9, 128.5, 128.9, 129.1, 129.2, 129.3, 129.4, 129.7, 130.4, 131.3, 134.1, 135.4, 137.7, 138.1, 141.8, 144.7, 162.4; MS (*m*/*z*, relative intensity %): 424.14 ($M^{+\cdot}$, 0.01), 287 (75), 270 (33), 244 (91), 243 (100), 242 (45).

*4.2.1.11. 1-(4-Methoxyphenyl)-*N′*-(4-methoxybenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**15**).* Yield: 78%; mp: 145–147 °C; IR (KBr) ν_{max} (cm^{-1}): 3283 (NH), 1672 (C=O), 1509–1304 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.86 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.98 (2H, d, $J = 7.0$ Hz, H-2″/H-6″), 7.18 (2H, d, $J = 8.7$ Hz, H-3′/H-5′), 7.35 (1H, d, $J = 7.5$ Hz, H-6), 7.57 (1H, d, $J = 7.5$ Hz, H-8), 7.60 (1H, td, $J = 7.5$ and 2.5 Hz, H-7), 7.79 (2H, d, $J = 8.7$ Hz, H-2′/H-6′),: 7.98 (2H, d, $J = 7.0$ Hz, H-3″/H-5″), 8.20 (1H, s, N=CH), 8.22 (1H, d, $J = 7.8$ Hz, H-5), 8.96 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 55.5, 55.4, 112.2, 114.2, 114.5, 114.7, 120.4, 120.8, 121.9, 126.6, 128.8, 129.4, 129.8, 130.3, 130.4, 135.1, 138.4, 141.2, 141.4, 148.3, 160.4, 161.5, 161.6; MS (*m*/*z*, relative intensity %): 450.10 ($M^{+\cdot}$, 8.1), 318 (22), 317 (100), 301 (15), 300 (45).

*4.2.1.12. 1-(4-Methoxyphenyl)-*N′*-(4-*N,N*-dimethylaminobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**16**).* Yield: 83%; mp: 310–312 °C; IR (KBr) ν_{max} (cm^{-1}): 3417 (NH), 1599 (C=O), 1514–1300 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.03 (6H, s, $N(CH_3)_2$), 3.93 (3H, s, OCH_3), 7.18 (2H, d, $J = 8.7$ Hz, H-3′/H-5′), 7.36 (1H, t, $J = 7.8$ Hz, H-6), 7.58 (1H, d, $J = 7.8$ Hz, H-8), 7.60 (1H, td, $J = 7.8$ and 2.0 Hz, H-7), 7.67 (2H, d, $J = 7.5$ Hz, H-3″/H-5″), 7.72 (2H, d, $J = 8.7$ Hz, H-2′/H-6′), 7.97 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 8.14 (1H, s, N=CH), 8.22 (1H, d, $J = 7.8$ Hz, H-5), 8.90 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 40.3, 55.7, 111.9, 112.2, 113.9, 114.6, 120.9, 121.5, 122.2, 126.8, 128.9, 129.4, 129.6, 129.8, 130.5, 138.0, 138.9, 141.3, 144.9, 149.4, 160.6, 161.2, 161.4; MS (*m*/*z*, relative intensity %): 463.12 ($M^{+\cdot}$, 29.0), 317 (79), 300 (41), 274 (100), 273 (50).

*4.2.1.13. 1-(4-Methoxyphenyl)-*N′*-(benzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**17**).* Yield: 72%; mp: 160–162 °C; IR (KBr) ν_{max} (cm^{-1}): 3283 (NH), 1671 (C=O), 1513–1320 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.91 (3H, s, OCH_3), 7.16 (2H, d, $J = 8.5$ Hz, H-3'/H-5'), 7.34 (1H, dd, $J = 8.0$ and 2.0 Hz, H-6), 7.41 (3H, m, Ar–H), 7.56 (1H, dd, $J = 8.0$ and 2.0 Hz, H-7), 7.60 (1H, d, $J = 8.0$ Hz, H-8), 7.80 (2H, d, $J = 7.0$ Hz, H-2″/H-6″), 7.97 (2H, d, $J = 8.5$ Hz, H-2′/H-6′), 8.17 (1H, d, $J = 8.0$ Hz, H-5), 8.23 (1H, s, N=CH), 8.85 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 55.6, 112.2, 114.7, 114.2, 120.9, 122.1, 122.2, 127.9, 128.8, 128.9, 129.8, 130.4, 130.5, 130.6, 133.9, 135.1, 138.5, 141.2, 141.4, 148.4, 160.5, 160.6; MS (*m*/*z*, relative intensity %): 420.08 ($M^{+\cdot}$, 6.4), 318 (21), 317 (100), 300 (47), 274 (96), 273 (66).

*4.2.1.14. 1-(4-Methoxyphenyl)-*N′*-(4-nitrobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**18**).* Yield: 68%; mp: 255–257 °C; IR (KBr) ν_{max} (cm^{-1}): 3326 (NH), 1675 (C=O), 1523–1309 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.96 (3H, s, OCH_3), 7.20 (2H, d, $J = 8.7$ Hz, H-3'/H-5'), 7.35 (1H, dd, $J = 7.5$; 2.5 Hz, H-6), 7.54 (1H, dd, 7.5 and 2.0 Hz, H-7), 7.62 (1H, d, $J = 7.5$ Hz, H-8), 8.00 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 8.03 (2H, d, $J = 8.7$ Hz, H-2′/H-6′), 8.23 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 8.29 (1H, d, $J = 8.7$ Hz, H-5), 8.42 (1H, s, N=CH), 8.98 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 55.2, 112.3, 114.0, 114.3, 120.6, 120.9, 121.6, 123.8, 128.2, 128.7, 129.7, 130.0, 130.1, 130.3, 135.7, 137.4, 140.2, 141.5, 145.5, 148.7, 150.3, 160.4; MS (*m*/*z*, relative intensity %): 465.06 ($M^{+\cdot}$, 3.9), 317 (100), 300 (42), 274 (95).

*4.2.1.15. 1-(3-Nitrophenyl)-*N′*-(4-methoxybenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**19**).* Yield: 80%; mp: 184–186 °C; IR (KBr) ν_{max} (cm^{-1}): 3297 (NH), 1664 (C=O), 1528–1305 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.84 (3H, s, OCH_3), 6.97 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 7.35 (1H, t, $J = 7.8$ Hz, H-6), 7.60 (1H, dd, $J = 7.8$ and 2.0 Hz, H-6′), 7.69 (1H, d, $J = 8.1$ Hz, H-8), 7.73 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 7.93 (1H, t, $J = 7.8$ Hz, H-7), 8.34 (1H, d, $J = 8.1$ Hz, H-5), 8.39 (1H, dd, $J = 7.5$; 3.0 Hz, H-4′), 8.57 (1H, s, N=CH), 8.63 (1H, d, $J = 8.7$ Hz, H-5′), 8.94 (1H, t, $J = 3.0$ Hz, H-2′), 8.97 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 54.9, 112.4, 113.9, 114.4, 120.3, 121.0, 121.6, 123.1, 123.5, 126.7, 128.6, 128.8, 129.8, 130.4, 134.6, 135.2, 138.2, 138.8, 138.9, 141.6, 148.1, 148.2, 160.7, 160.8; MS (*m*/*z*, relative intensity %): 467.10 ($M^{+\cdot}$, 0.5), 332 (75), 289 (100), 288 (36), 261 (41), 242 (68).

*4.2.1.16. 1-(3-Nitrophenyl)-*N′*-(4-*N,N*-dimethylaminobenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**20**).* Yield: 85%; mp: 260–262 °C; IR (KBr) ν_{max} (cm^{-1}): 3492 (NH), 1655 (C=O), 1522–1321 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.04 (6H, s, $N(CH_3)_2$), 6.72 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 7.35 (1H, t, $J = 7.8$ Hz, H-6), 7.60 (1H, t, $J = 7.8$ Hz, H-7), 7.66 (1H, d, $J = 7.8$ Hz, H-8), 7.68 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 7.88 (1H, dd, J = 7.8 and 2.0 Hz, H-6′), 7.90 (1H, d, $J = 8.1$ Hz, H-4′), 8.29 (1H, d, $J = 7.8$ Hz, H-5), N=CH: 8.38 (1H, s, N=CH), 8.58 (1H, d, J = 8.1 Hz, H-5′), 8.97 (1H, s, H-4), 8.94 (1H, t, $J = 2.0$ Hz, H-2′), 11.42–11.86 (2H, s, NH); ^{13}C NMR (DMSO-d_6): δ 56.8, 110.7, 111.8, 113.7, 119.8, 120.5, 120.6, 120.9, 122.5, 123.0, 128.1, 128.2, 129.1, 130.0, 134.1, 134.4, 137.6, 138.3, 138.5, 141.1, 147.6, 148.4, 150.8, 160.0; MS (*m*/*z*, relative intensity %): 480.06 ($M^{+\cdot}$, 1.3), 289 (76), 243 (46), 242 (70), 241 (38), 146 (100).

*4.2.1.17. 1-(4-Nitrophenyl)-*N′*-(4-methoxybenzylidene)-9*H*-pyrido[3,4-b]indole-3-carbohydrazide (**21**).* Yield: 76%; mp: 166–168 °C; IR (KBr) ν_{max} (cm^{-1}): 3288 (NH), 1668 (C=O), 1512–1309 (C=C); ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.81 (3H, s, OCH_3), 7.05 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 7.35 (1H, t, $J = 7.0$ Hz, H-6), 7.64 (1H, t, $J = 7.0$ Hz, H-7), 7.70 (1H, d, $J = 7.0$ Hz, H-8), 7.75 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 8.45 (2H, d, $J = 7.0$ Hz, H-3′/H-5′), 8.50 (1H, d, $J = 7.0$ Hz, H-5), 8.55 (2H, d, $J = 7.0$ Hz, H-2′/H-6′), 8.61 (1H, s, N=CH), 9.02 (1H, s, H-4); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 55.9, 112.7, 114.4, 115.0, 120.9, 121.1, 122.4, 123.7, 127.0, 128.7, 130.3, 130.6, 134.7, 138.2, 139.4, 139.9, 141.7, 142.5, 143.6, 147.5, 148.4, 160.8; MS (*m*/*z*, relative

intensity %): 467.03 ($M^{+\cdot}$, 0.5), 332 (82), 288 (100), 287 (40), 243 (43), 242 (80).

*4.2.1.18. 1-(4-Nitrophenyl)-N′-(4-N,N-dimethylaminobenzylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide (**22**).* Yield: 80%; IR (KBr) ν_{max} (cm^{-1}): 3420 (NH), 1660 (C═O), 1519–1322 (C═C); ^1H NMR (300 MHz, DMSO-d_6): δ 2.98 (3H, s, CH_3), 6.76 (2H, d, $J = 8.7$ Hz, H-3″/H-5″), 7.35 (1H, t, $J = 7.0$ Hz, H-6), 7.59 (2H, d, $J = 8.7$ Hz, H-2″/H-6″), 7.63 (1H, t, $J = 7.0$ Hz, H-7), 8.23 (2H, d, $J = 7.5$ Hz, H-3′/H-5′), 8.47 (2H, d, $J = 7.5$ Hz, H-2′/H-6′), 8.51 (1H, s, N═CH), 8.48 (1H, d, $J = 7.0$ Hz, H-5), 9.01 (1H, s, H-4), 12.12–1159 (2H, s, N–H). ^{13}C NMR (DMSO-d_6): δ 60.3, 111.8, 112.7, 114.8, 120.6, 121.0, 122.4, 123.8, 128.5, 129.6, 130.3, 130.7, 134.7, 137.2, 138.1, 138.2, 139.7, 141.7, 147.5, 149.3, 159.4, 160.5. MS (*m*/*z*, relative intensity %): 478.03 ($M^{+\cdot}$, 4.5), 332 (79), 288 (100), 287 (40), 242.00 (78).

*4.2.1.19. 1-(4-Nitrophenyl)-N′-(benzylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide (**23**).* Yield: 73%; mp: 220–222 °C; IR (KBr) ν_{max} (cm^{-1}): 3279 (NH), 1664 (C═O), 1521–1324 (C═C); ^1H NMR (300 MHz, DMSO-d_6): δ 7.36 (1H, t, $J = 7.5$ Hz, H-6), 7.44–7.51 (5H, m, Ar–H), 7.66 (1H, ddd, $J = 7.0$, 7.5 and 1.5 Hz, H-7), 7.70 (1H, d, $J = 7.5$ Hz, H-8), 7.78 (2H, d, $J = 8.1$ Hz, H-3′/H-5′), 8.50 (2H, d, $J = 8.0$ Hz, H-2′/H-6′), 8.54 (1H, d, $J = 7.8$ Hz, H-5), 8.67 (1H, s, N═CH), 9.04 (1H, s, H-4), 12.14–11.89 (2H, s, N–H); ^{13}C NMR (DMSO-d_6): δ 112.7, 115.2, 120.7, 121.0, 122.4, 123.8, 127.1, 128.9, 129.2, 130.1, 130.4, 130.7, 134.5, 134.8, 139.3, 139.7, 141.7, 143.5, 147.5, 148.5, 161.1; MS (*m*/*z*, relative intensity %): 435.06 ($M^{+\cdot}$, 5.3), 332.01 (87.5), 289.02 (100), 242.01 (87.4).

4.3. Antiviral assay

4.3.1. Screening tests

Vero cells monolayers, infected or not with poliovirus (PV) or herpesvirus (HSV-1), were treated with different concentrations of each synthesized compounds and the antiviral activity was detected by Sulforhodamine B assay (SRB) [32]. The cytotoxicity was evaluated against Vero cells after 72 h of drug exposure using the SRB assay. EC_{50}, CC_{50} and SI were determined and the results of all tested compounds are listed in Table 1.

4.3.2. Virus yield inhibition assay

4.3.2.1. Effect on viral adsorption. Compounds were dissolved in DMSO, diluted in serum free DMEM at different concentrations and incubated with Vero cell monolayer pre-washed with phosphate-buffered saline (PBS) in 24-well tissue culture plates for 1 h at 37 °C in 5% CO_2. After removal of the unbound compound, the cells were washed with PBS and then infected with 40–50 UFP/well of HSV-1 virus. After incubating for 1 h, the unabsorbed virus was removed; the cell monolayer was washed with PBS and then incubated in DMEM containing 0.5% carboxymethylcellulose.

4.3.2.2. Treatment during infection. The assay was performed as described above, with the exception that the compound was added together with the virus. After 1 h incubation, the solution containing unabsorbed virus was removed, the cell monolayer was washed with PBS and further incubated in DMEM containing 0.5% carboxymethylcellulose.

4.3.2.3. Treatment after infection. To evaluate the effect of the compounds on infected cells, monolayer Vero cells were washed with PBS and infected. After 1 h incubation, the unabsorbed virus was removed and the cell monolayer was washed with PBS and then incubated with different concentrations of the compounds in DMEM containing 0.5% carboxymethylcellulose.

4.3.2.4. Virucidal activity. The viral suspensions were pre-incubated with different concentrations at 37 °C for 1 h. The mixtures were used to infect Vero cells for 1 h at 37 °C in 5% CO_2. Next, the cell monolayer was washed with PBS and further incubated in DMEM containing 0% carboxymethylcellulose.

In all of the experiments above, after 48 or 72 h incubation at 37 °C, cell monolayers were fixed with 10% formaldehyde, stained with solution of 0.5% crystal violet and the number of plaques was counted. Controls consisted of either untreated Vero cells or infected Vero cells.

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References

[1] D.M. Knipe, P.M. Howley (Eds.), Fields Virology, vol. 2, Lippincott Williams and Wilkins, Philadelphia, PA, 2001, p. 2381.
[2] J. Benedetti, L. Corey, R. Ashley, Ann. Intern. Med. 121 (1994) 847–854.
[3] P.A. Furman, P. De Miranda, M.H.S. De Clair, G.B. Elion, Antimicrob. Agents Chemother. 20 (1981) 518–524.
[4] F. Morfin, D. Thouvenot, J. Clin. Vir. (2003) 29–37.
[5] S. Mueller, E. Wimmer, J. Cello. Virus Res. 111 (2005) 175–193.
[6] J. Ishida, H. Wang, M. Oyama, M.L. Cosentino, C. Hu, K. Lee, J. Nat. Prod. 64 (2001) 958–960.
[7] Z.J. Dong, F. Wang, R.R. Wang, L.M. Yang, Y.T. Zheng, J.K. Liu, Chin. Tradit. Herb. Drugs 38 (2007) 17–19.
[8] J.G. Tang, Y.H. Wang, R.R. Wang, Z.J. Dong, L.M. Yang, Y.T. Zheng, J.K. Liu, Chem Biodivers. 5 (2008) 447–460.
[9] X. Yu, W. Lin, J. Li, M. Yang, Eur. J. Med. Chem. 40 (2005) 831–839.
[10] H. Guan, H. Chen, W. Peng, Y. Ma, R. Cao, X. Liu, A. Xu, Eur. J. Med. Chem. 41 (2006) 1167–1179.
[11] L.T.D. Tonin, V.A. Barbosa, C.C. Bocca, E.R.F. Ramos, C.V. Nakamura, W.F. Costa, E.A. Basso, T.U. Nakamura, M.H. Sarragiotto, Eur. J. Med. Chem. 44 (2009) 1745–1750.
[12] Y.C. Shen, C.Y. Chen, P.W. Hsieh, C.Y. Duh, Y.M. Lin, C.L. Ko, Chem. Pharm. Bull. 53 (2005) 32–36.
[13] R. Cao, W. Peng, H. Chen, X. Hou, H. Guan, Q. Chen, Y. Ma, A. Xu, Eur. J. Med. Chem. 40 (2005) 249–257.
[14] J. Ishida, H.K. Wang, K.F. Bastow, C.Q. Hu, K.H. Lee, Bioorg. Med. Chem. Lett. 9 (1999) 3319–3324.
[15] R. Cao, H. Chen, Y. Ma, X. Hou, H. Guan, X. Liu, A. Xu, Eur. J. Med. Chem. 40 (2005) 991–1001.
[16] R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A. Xu, Bioorg. Med. Chem. 12 (2004) 4613–4623.
[17] M. Zhao, L. Bi, W. Wang, C. Wang, M. Baudy-Floc'h, J. Ju, S. Peng, Bioorg. Med. Chem. 14 (2006) 6998–7010.
[18] R. Cao, W. Peng, H. Chen, Y. Ma, X. Liu, X. Hou, H. Guan, A. Xu, Biochem. Biophys. Res. Commun. 338 (2005) 1557–1563.
[19] Q. Wu, R. Cao, M. Feng, X. Guan, C. Ma, J. Liu, H. Song, W. Peng, Eur. J. Med. Chem. 44 (2009) 533–540.
[20] L.Q. Al-Mawsawi, R. Dayam, L. Taheri, M. Witvrouw, Z. Debyser, N. Neamati, Bioorg. Med. Chem. Lett. 17 (2007) 6472–6475.
[21] M. Marastoni, A. Baldisserotto, C. Trapella, J. McDonald, F. Bortolotti, R. Tomatis, Eur. J. Med. Chem. 40 (2005) 445–451.
[22] R. Ragno, M. Artico, G. De Martino, G. La Regina, A. Coluccia, A. Di Pasquali, R. Silvestri, J. Med. Chem. 48 (2005) 213–223.
[23] R. Ragno, A. Coluccia, G. La Regina, G. De Martino, F. Piscitelli, A. Lavecchia, E. Novellino, A. Bergamini, C. Ciaprini, A. Sinistro, G. Maga, E. Crespan, M. Artico, R. Silvestri, J. Med. Chem. 49 (2006) 3172–3184.
[24] P.D. Bailey, S.P. Hollinshead, N.R. McLay, Tetrahedron Lett. 28 (1987) 5177–5180.
[25] S.K. Srivastava, A. Agarwal, P.M.S. Chauhan, S.K. Agarwal, A.P. Bhaduri, S.N. Singh, N. Fatima, R.K. Chatterjee, Bioorg. Med. Chem. 7 (1999) 1223–1236.
[26] E. Wimmer, C.U. Hellen, X. Cao, Annu. Rev. Genet. 27 (1993) 353–436.
[27] Y. Zhao, M.H. Abraham, J. Lee, A. Hersey, N.Ch. Luscombe, G. Beck, B. Sherborne, I. Cooper, Pharm. Res. 19 (2002) 1446–1457.
[28] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[29] I.V. Tetko, Drug Discov. Today 10 (2005) 1497–1500.
[30] M. Marchetti, S. Pisani, G. Antonini, P. Valenti, L. Seganti, N. Orsi, Biometals 11 (1998) 89–94.
[31] U. Lindahl, K. Lidholt, D. Spillmann, L. Kjellén, Thromb. Res. 75 (1994) 1–32.
[32] P. Skehan, Assays of cell growth and cytotoxicity, in: Studzinski (Ed.), Cell Growth and Apoptosis: A Practical Approach, Oxford University Press, 1995, p. 169.