The Effects of the Diterpenes Isolated from the Brazilian Brown Algae *Dictyota pfaffii* and *Dictyota menstrualis* against the Herpes Simplex Type-1 Replicative Cycle

Authors Juliana L. Abrantes[1], Jussara Barbosa[2], Diana Cavalcanti[2], Renato C. Pereira[2], Carlos Frederico L. Fontes[1], Valeria L. Teixeira[2], Thiago Moreno L. Souza[4*], Izabel C. P. Paixão[3*]

Affiliations The affiliations are listed at the end of the article

Key words
- Dictyotaceae
- *Dictyota menstrualis*
- *Dictyota pfaffii*
- marine algae
- diterpenes
- antiviral
- HSV-1

Abstract

We describe in this paper that the diterpenes 8,10,18-trihydroxy-2,6-dolabelladiene (**1**) and (6*R*)-6-hydroxydichotoma-4,14-diene-1,17-dial (**2**), isolated from the marine algae *Dictyota pfaffii* and *D. menstrualis*, respectively, inhibited HSV-1 infection in Vero cells. We initially observed that compounds **1** and **2** inhibited HSV-1 replication in a dose-dependent manner, resulting in EC_{50} values of 5.10 and 5.90 µM, respectively, for a multiplicity of infection (MOI) of 5. Moreover, the concentration required to inhibit HSV-1 replication was not cytotoxic, resulting in good selective index (SI) values. Next, we found that compound **1** sustained its anti-herpetic activity even when added to HSV-1-infected cells at 6 h after infection, while compound **2** sustained its activity for up to 3 h after infection, suggesting that these compounds inhibit initial events during HSV-1 replication. We also observed that both compounds were incapable of impairing HSV-1 adsorption and penetration. In addition, the tested molecules could decrease the contents of some HSV-1 early proteins, such as UL-8, RL-1, UL-12, UL-30 and UL-9. Our results suggest that the structures of compounds **1** and **2**, Brazilian brown algae diterpenes, might be promising for future antiviral design.

Introduction

Herpes viruses are a large and diverse family of enveloped viruses composed of three subfamilies: alpha-, beta-, and gamma-herpes viruses. Herpes simplex virus type 1 (HSV-1) is a ubiquitous alpha-herpes virus, whose virions consist of a large, double-stranded DNA genome packaged within an icosahedral capsid encased by a proteinaceous tegument and a lipidic envelope composed of various glycoproteins [1].

The HSV-1 replication cycle has been well characterized [2]. HSV-1 entry into cells is a complex process that involves an initial attachment of virus glycoproteins to cell surface receptors [3]. Once inside the cell, the HSV-1 replication cycle can be divided into three phases. First, HSV-1 immediate-early (IE; α) gene expression depends on the binding of the α-*trans*-induction factor (αTIF)/C1/Oct-1 multiprotein complex to the TAAGARAT (R, purine; GARAT) sequences of the *cis*-acting site in the HSV-1 genome [4]. Consequently, viral IE proteins, such as infected-cell protein 0 (ICP0), ICP27 and ICP4, regulate subsequent steps of viral replication. Second, early (E; β) genes, such as viral helicase (UL-8), DNA polymerase (UL-30) and thymidine kinase, are expressed and viral DNA is synthesized. Last, late (L; γ) genes encoding for structural proteins such as gB, gC and ICP5 are expressed [2]. It has been proposed that inhibition of any of these steps may lead to the blockage of HSV-1 replication [5,6].

After primary infection of mucosal tissues, HSV-1 establishes latency and can be reactivated under several conditions, causing a variety of infections, principally in individuals immunocompromised by AIDS, cancer, or organ transplantation [7]; this reactivation is considered to be an important event associated with increased HIV transmission [8].

Several anti-herpetic agents have been extensively investigated and used in the treatment of HSV-1-infected individuals, including acyclovir and penciclovir, and their prodrugs valacyclovir and famciclovir [9]. However, long-term administration of these drugs to immunocompromised patients may result in the emergence of drug-re-

received April 10, 2009
revised August 9, 2009
accepted August 19, 2009

Bibliography
DOI 10.1055/s-0029-1186144
Published online September 17, 2009
Planta Med 2010; 76: 339–344

ISSN 0032-0943

Correspondence
Juliana Lourenço Abrantes, BSc, MSc
Instituto de Bioquímica Médica
Cidade Universitária
Ilha do Fundão
Centro de Ciências da Saúde (CCS)
Bloco H, segundo andar, sala 26
Rua Cesar Pernetta 400
CEP 21941–590, Rio de Janeiro
Brasil
Phone: + 55 21 25 62 67 84
Fax: + 55 21 25 62 67 84
abrantes@bioqmed.ufrj.br

* Both authors contributed equally to this paper.

sistant HSV-1 isolates. Therefore, the search for new antiviral agents, especially with different mechanisms of action, is a crucial goal [10].

Polysaccharides, alkaloids, phenolic compounds, terpenoids, steroids and peptides are examples of non-nucleoside natural products reported as inhibitors of HSV-1 replication [12,13]. We have previously described the anti-HSV-1 effects of alkaloids isolated from a marine sponge [14] and of diterpenes isolated from the Brazilian brown alga *D. pfaffii* [15,16]. The anti-HIV-1 activities of products derived from this alga have also been reported, together with the effects of molecules from another brown alga, *D. menstrualis* [15–20]. In our previous work, we demonstrated the inhibition of HSV-1 cytopathic effects by diterpenes isolated from *D. pfaffii*, as well as their cytotoxicity toward Vero cells [15]. However, the mechanism of action by which HSV-1 replication was inhibited remained to be elucidated. Thus, in order to understand the mode of action of the main active product from *D. pfaffii* and to investigate the anti-HSV-1 activity of another diterpene isolated from *D. menstrualis*, we performed *in vitro* studies to determine the EC_{50} and CC_{50} of these natural products, as well as their effects on HSV-1 adsorption, penetration and the virus replication cycle. We found that the studied compounds were potent inhibitors of HSV-1 replication endowed with the ability to diminish the content of important early viral proteins.

Materials and Methods

Compounds

The diterpenes 8,10,18-trihydroxy-2,6-dolabelladiene (**1**) and (6*R*)-6-hidroxydichotoma-4,14-diene-1,17-dial (**2**) (**Fig. 1**) were obtained as described previously [15,17]. These molecules were isolated from the Brazilian brown algae *D. pfaffii* and *D. menstrualis*, respectively, and tested for purity as described elsewhere [16,21]. Both algae were collected at the Brazilian shore; *D. pfaffii* was collected at Atol das Rocas, Rio Grande do Norte, BR, and *D. menstrualis* at Buzios, Rio de Janeiro, BR, and they were identified by V.L. Teixeira, one of the authors of this paper. The algae were deposited at the Herbarium of the University of Rio de Janeiro, Brazil (HBRJ), with the voucher numbers HBRJ10017 and HBRJ9117 for *Dictyota menstrualis* and *Dictyota pfaffii*, respectively. The diterpenes, at over 99% purity, were diluted in 100% dimethyl sulfoxide (DMSO) and stored at − 20 °C. The resulting DMSO concentrations during the assays were below 0.1%, a level that is not significantly cytotoxic.

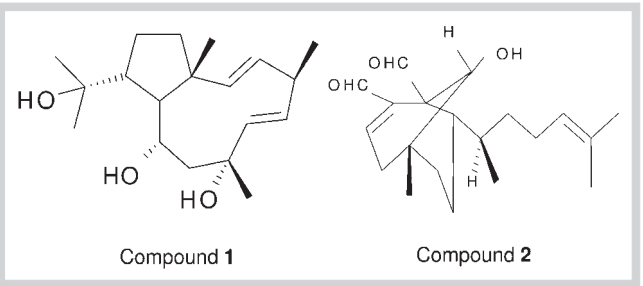

Fig. 1 The chemical structures of compounds **1** and **2** [16,21].

Cells and virus

African green monkey kidney cells (Vero cells; ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% fetal bovine serum (FBS; HyClone), 100 U/mL penicillin, and 100 µg/mL streptomycin and incubated at 37 °C in 5% CO_2. In order to prepare virus stocks, Vero cells were infected with HSV-1 (AR-29 strain) [22] at a multiplicity of infection (MOI) of 0.1. At 24 h postinfection (*p.i.*), the cells were lysed by three cycles of freezing and thawing, centrifuged at 1500 × *g* at 4 °C for 20 min, and the supernatant was collected and stored at − 70 °C for further studies [23].

Cytotoxicity assay

Monolayers of Vero cells in 96-multiwell plates (1×10^4/well) were treated with either compound **1** or **2** for 72 h, and 50 µL of a 1 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) diluted in DMEM without serum was then added to the cells. MTT was removed after 3 h, 50 µL of acid-isopropyl alcohol (0.04 N HCl in isopropyl alcohol) was added and the optical density (OD) was read using an automatic plate reader with a 570 nm test wavelength and a 690 nm reference wavelength [24]. The cytotoxic concentration in 50% (CC_{50}) was calculated by linear regression analysis of the dose-response curves generated from the data using Excel for Windows software.

Plaque-forming assay

Monolayers of Vero cells in 6-well plates (3×10^5/well) were exposed to supernatant from the yield reduction assay for 1 h at 37 °C. Next, residual viruses were removed, and DMEM containing 5% FBS and 1% methylcellulose (Fluka) (overlay medium) was added to cells. After 72 h at 37 °C, the monolayers were fixed with 10% formaldehyde in PBS and stained with a 0.1% solution of crystal violet in 70% methanol, and the virus titers were calculated by scoring the plaque-forming units (PFU) [25].

Yield reduction assay

Monolayers of Vero cells in 24-well plates (1×10^5/well) were infected with HSV-1 at a MOI of 1 or 5 for 1 h at 37 °C. Cells were washed and the compounds were added at various concentrations in DMEM with 5% FBS. After 20 h, the cells were lysed, cellular debris were cleared by centrifugation, and virus titers in the supernatant were determined by a plaque-forming assay using Vero cells. In every assay, we included acyclovir (ACV; Sigma, > 99% pure) as a control. The antiviral activity at 50% (EC_{50}) was measured by the linear regression analysis of the dose-response curve mentioned above, using Excel for Windows software.

Adsorption and penetration inhibition assays

In order to evaluate whether compound **1** or **2** could inhibit virus adsorption, Vero cells in 6-well plates were exposed to 100 PFU of HSV-1/well and treated with the EC_{50} of these compounds at 4 °C. Next, cells were washed and covered with overlay medium, and the temperature was raised to 37 °C for 72 h [26], after which plaques were analyzed as described in the plaque-forming assay. For this kind of assay, heparin (Sigma; > 99% pure) was used as a control.

In order to measure HSV-1 penetration, we used the protocol mentioned above with some modifications. In brief, the same number of Vero cells was incubated with 100 PFU/well of HSV-1 at 4 °C for 1 h. Next, the temperature was raised to 37 °C in the presence of compound **1** or **2** and, 1 h after that, cells were washed with PBS containing glycine at pH 2.2. Cells were then

Compounds	CC_{50}	EC_{50}		SI*	
		MOI = 1	MOI = 5	MOI = 1	MOI = 5
1	184 ± 21**	1.20 ± 0.04	5.10 ± 0.03	153	36
2	1000 ± 83	1.60 ± 0.08	5.90 ± 0.20	625	169
ACV	860 ± 32	1.20 ± 0.10	6.50 ± 0.06	716	132

Table 1 Cytotoxicity and antiviral activity of the natural products derived from the algae *Dictyota pfaffii* and *Dictyota menstrualis*.

* The selective index (SI) was calculated based on the ratio between CC_{50} and EC_{50}. ** This value was obtained from our previous work [15]

covered with overlay medium and plaques were counted after 72 h of infection [14].

Time-of-addition experiments

In order to analyze whether addition of compound **1** or **2** could be delayed without losing their ability to block HSV-1 replication, and to better understand at what moment of the HSV-1 replication cycle these compounds might be acting, we performed a time-of-addition assay. Vero cells in 6-well plates were infected with 100 PFU/well for 1 h and washed, and the compounds were added at 10 µM (2 × EC_{50}) at different times *p.i.* [26]. At the same time that compound **1** or **2** was added, cells were also covered with overlay medium and, after 72 h *p.i.*, plaques were counted. As a control, we also performed additional assays with the reference compound acyclovir.

HSV-1 protein synthesis assay

In order to understand whether compounds **1** and **2** were able to interfere in HSV-1 protein synthesis, we performed pulse-and-chase experiments. Vero cells in 25 cm^2 bottles (2×10^6 cells) were infected with HSV-1 at a MOI of 5 or 20 for 1 h at 37 °C. Next, residual viruses were removed and the cells were treated with compound **1** or **2** at 10 µM for 4 h. After this period, the cells were replenished with methionine-free DMEM in the presence of the compounds, supplemented with [^{35}S]-methionine (50 µCi/mL) for 2 h. Then, the cells were lysed with buffer A (1 M Tris-HCl, pH 6.8; 0.02% bromophenol blue; 5% β-mercaptoethanol; 10% SDS and 10% glycerol). These cell lysates were counted by liquid phase scintillation (Packard; model TRI-CARB 2100 TR) and 20 µg of the crude cell extracts, derived from assays in which we used a MOI of 20, were analyzed by SDS-PAGE electrophoresis, as described previously [27]. The protein electrophoresis was performed at least four times and the results are displayed as a representative gel. In every assay performed, an identical protein profile was seen; thus, p values for the representative proteins that had their content impaired were < 0.001.

Statistical analysis

All data presented in this paper represent the means ± standard error of the means (SEM) of at least three independent experiments. All statistical analyses were performed using Excel for Windows and p values equal to or less than 0.05 were considered statistically significant by Student's t-test.

Results and Discussion

Since macro- and microalgae represent a fruitful source of natural products [11, 28], and considering that we have previously described the antiviral activity of the diterpenes derived from two distinct Brazilian brown algae, *D. pfaffii* and *D. menstrualis* [15–20], we further explored the mode of inhibition of the main compounds of these algae, compounds **1** and **2**, against HSV-1 replication. Initially, we analyzed the effects of compound **1** toward this virus's replication and found a potent dose- and MOI-dependent effect of this molecule, resulting in an EC_{50} equal to 1.2 µM with respect to an MOI of 1 and 5.1 µM when tested at an MOI of 5, with low cytotoxicity (CC_{50} = 184; ▶ **Table 1**). Based on the ratio of CC_{50} and EC_{50}, we calculated the selective index (SI) values, which were equal to 153 and 36 at MOIs of 1 and 5, respectively.

Moreover, we also showed that another brown alga natural product, the diterpene compound **2** derived from *D. menstrualis*, was also able to inhibit HSV-1 replication in Vero cells (▶ **Table 1**). Once more, we observed a pronounced dose- and MOI-dependent inhibition. The resulting EC_{50} was equal to 1.6 µM and 5.9 µM at MOIs of 1 and 5, respectively (▶ **Table 1**). Remarkably, compound **2** produced very low cytotoxicity in Vero cells, as we observed a CC_{50} equal to 1000 µM (▶ **Table 1**). As a result of this difference between the CC_{50} and EC_{50} for compound **2**, a high SI was seen, with values of 625 and 169.4 for MOIs of 1 and 5, respectively.

For comparison, the effect of the reference compound ACV was also studied under our assay conditions. Importantly, the EC_{50} values for acyclovir were 1.2 µM and 6.5 µM for the MOIs of 1 and 5, respectively (▶ **Table 1**). The results displayed in ▶ **Table 1** also show that under high viral doses, the natural products were slightly more potent than ACV. Due to the low cytotoxicity of ACV, the SI values for the reference compound were higher than those observed for compound **1**, but comparable to that seen for compound **2** (▶ **Table 1**). Therefore, due to the higher cytotoxicity of compound **1**, this molecule could be considered less potent than ACV and its brown alga counterpart. Nevertheless, since compound **1**'s EC_{50} values were comparable to both compound **2** and the reference compound – and considering its structural singularity – we decided to continue our investigation with compound **1** as well. Interestingly, among natural products, ours were less cytotoxic than other products described elsewhere [29]. The cytotoxicity of ACV was also examined in parallel, and we found a CC_{50} equal to 860 µM (▶ **Table 1**).

An additional advantage of our compounds is that both compounds **1** and **2** are inhibitors of HIV replication [15–20]. Therefore, since HSV-1 is an opportunistic pathogen found in HIV-infected individuals [8], our compound formulations could be potentially interesting as leads to the synthesis and/or the search for novel molecules able to simultaneously target the replication of both HIV-1 and HSV-1. This, in the future, from a more pragmatic point of view, might also reduce the number of drugs taken by HIV-infected persons.

Next, in order to gain insight into the stage at which compounds **1** and **2** were acting, we performed time-of-addition assays. Thus, HSV-1-infected Vero cells were treated for different times with the compounds at twofold their EC_{50} values, with respect to an MOI of 5. Such a dose was used to reach maximum inhibition while preventing nonspecific effects. In this assay, we chose the

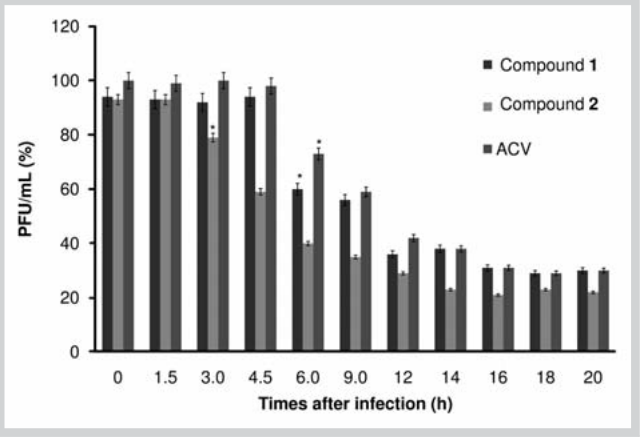

Fig. 2 Time-of-addition assay. Vero cells were infected with HSV-1, and either compound **1**, compound **2** (both at 10 µM) or acyclovir (14.5 µM) was added at different times after infection, as indicated. HSV-1 replication was measured by a plaque-forming assay 72 h *p. i.* Data represent means ± SEM of three independent experiments. The p values of < 0.05 can be noticed for compound **2** from 3 h *p. i.*, while for compound **1** and ACV it is observed at 6 h *p. i.*

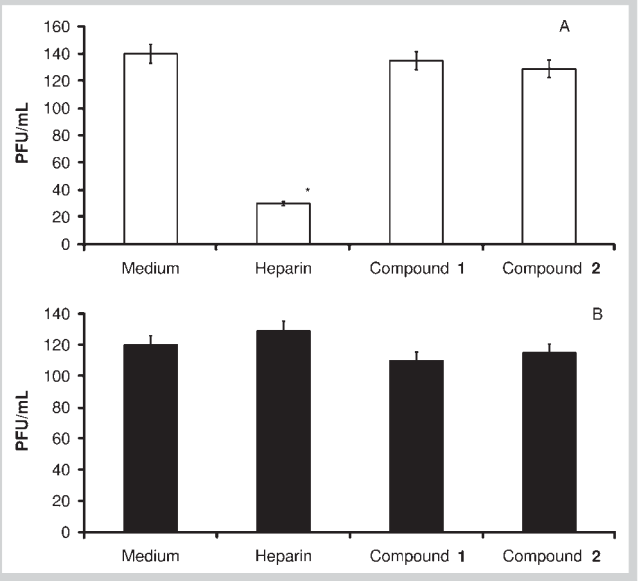

Fig. 3 Effects of compounds **1** compound **2** on HSV-1 adsorption and penetration. Vero cells were exposed to HSV-1 and treated with the EC_{50} values of compound **1** or **2**, or heparin at 1 µg/mL. **A** These procedures were performed at 4 °C to allow only virus adsorption for 1 h. After this period, cells were washed with PBS to remove unbound viruses, the temperature was raised to 37 °C to allow virus penetration and cells were covered with overlay medium. **B** Vero cells were exposed to HSV-1 at 4 °C for 1 h to allow only virus adsorption. Cells were then washed with PBS to remove unbound viruses, and the temperature was raised to 37 °C. Immediately after, cells were treated with the mentioned compounds; thus, virus penetration was only occurring in the presence of the compounds. After 1 h, cells were washed with PBS-glycine pH 2.2 to inactivate viruses that did not penetrate and were covered with overlay medium. After 72 h, virus replication, which was directly proportional to virus adsorption (**A**) or penetration (**B**), was measured by a plaque-forming assay. Data represent means ± SEM of four independent assays. The asterisks represent p < 0.05.

periods of drug addition based on the kinetics of the HSV-1 replicative cycle. For each chosen time, there is a peak of HSV-1 protein synthesis, characteristic of each step of HSV-1 replication [2]. In addition, cells were treated from 0 to 20 h *p. i.*, because one complete HSV-1 replicative cycle lasts for this period [2]. We found that the anti-herpetic activities of compounds **1** and **2** were preserved when these drugs were added to HSV-1-infected cells up to 6 h and 3 h after infection, respectively, declining thereafter (**Fig. 2**). This result suggests that these diterpenes inhibit initial events during HSV-1 replication. For comparison, addition of acyclovir, which inhibits the early phase of HSV-1 replication, could be delayed up to 6 h after infection. This result suggests that compound **1** and acyclovir might share similar kinetics of inhibition, while compound **2** and the reference compound have different mechanisms of action. Nevertheless, these observations, together with other data in the literature [23, 26], raise at least three possible targets for our compounds: viral adsorption, viral penetration and the initial steps of the HSV-1 replication process.

In order to further investigate our natural products' mechanisms of action, we performed adsorption and penetration assays. Initially, we analyzed the effects of compounds **1** and **2** on viral adsorption. For this purpose, Vero cells were infected and treated at 4 °C, since at this temperature, viruses bind but they do not enter the cells. When the temperature was raised to 37 °C, only bound viruses could enter; thus, plaque formation after 72 h *p. i.* was directly proportional to HSV-1 adsorption. We noticed that the natural compounds did not affect viral adsorption (**Fig. 3A**). For comparison, we used heparin as a positive control at 1 µg/mL; this concentration could inhibit ~ 80% of HSV-1 adsorption, as has been shown previously [30].

Second, we investigated the effects of compounds **1** and **2** on HSV-1 penetration. Vero cells were infected with HSV-1 at 4 °C, the temperature was raised to 37 °C, and the test compounds were immediately added to the infected cells. At 4 °C, viruses would only bind but they could not enter; when the temperature was raised, viruses would penetrate in the presence of the compound. Therefore, the plaque formation after 72 h was directly proportional to virus penetration. We failed to observe (**Fig. 3B**) HSV-1 penetration inhibition by the natural compounds.

Since our natural products inhibit HSV-1 at an initial event during its replication, but after viral adsorption/penetration, and considering that it has been described that HSV-1 replication leads to a shut-off of cellular protein synthesis [2], we took advantage of this viral feature to get insight into which viral proteins would have their concentration impaired upon treatment with compound **1** or **2**. Vero cells were infected for 1 h at 37 °C and were then treated with the compounds at two times their EC_{50} values. We observed (**Fig. 4A**) that HSV-1 infection could reduce Vero cell protein content. This event was prevented when the HSV-1-infected Vero cells were treated with either compound **1** or **2**. We also noticed that mock-infected cells treated with the natural products had a transient reduction of their protein content; this is probably a transient event because the drug concentration used in this assay is not cytotoxic (**Fig. 4A**). Additionally, to further qualitatively analyze the proteins in HSV-1-infected cells, we performed this same assay using an MOI of 20. This MOI was chosen since it produces a strong inhibition of cellular protein synthesis and, thus, might be useful to evaluate the main targets of our compounds on HSV-1 replication. As shown in **Fig. 4B** and **C**, the compounds did not change the protein content of mock-infected cells (lanes 1 and 2 of both panels). In

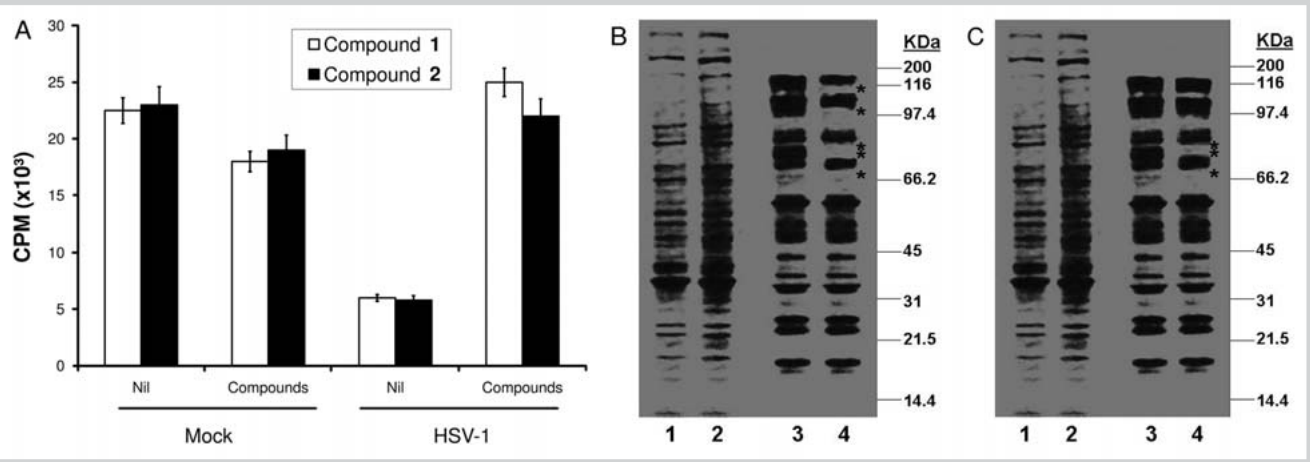

Fig. 4 Effects of the diterpenes compounds **1** and **2** on some viral proteins. Vero cells were infected with HSV-1 at an MOI of 5 and treated with compound **1** or **2** at 10 µM for 4 h. The cells were then replenished with DMEM containing [^{35}S]-methionine (50 µCi/mL) for 2 h. Cells lysates were counted by liquid phase scintillation (***A***). Lysates from HSV-1-infected Vero cells (MOI of 20) were also analyzed by SDS-PAGE electrophoresis and the gels were revealed by autoradiography (**B** and **C**). In panel **A**, data represent means ± SEM of four independent experiments and the asterisks represent $p < 0.05$. Panels **B** and **C** display representative gels with the following lanes: 1 – mock-infected Vero cells, 2 – mock-infected Vero cells treated with compound **1** (**B**) or compound **2** (**C**), 3 – HSV-1-infected Vero cells and 4 – HSV-1-infected Vero cells treated with compound **1** (**B**) or compound **2** (**C**). The molecular weights of the diminished proteins UL-30, UL-9, UL-8, UL-12 and RL-1 are 115.32, 94.26, 79.92, 67.50 and 66.19 KDa, respectively.

addition, in panels **B** and **C** of **Fig. 4**, we also observed that HSV-1 infection reduced the protein content of Vero cells (lanes 1 and 3). Interestingly, compound **1** diminished the levels of some viral proteins, including UL-30 (HSV-1 DNA polymerase; 115.32 KDa) and UL-9 (ori-binding protein; 94.26 KDa) (**Fig. 4B**; lanes 3 and 4). This effect was shared, in part, with compound **2**, since the concentrations of the proteins UL-8 (viral helicase; 79.92 KDa), RL-1 (a modulator of cell cycle and viral gene expression; 66.19 KDa) and UL-12 (a viral deoxyribonuclease; 67.50 KDa) were also inhibited by this molecule (**Fig. 4C**, lanes 3 and 4). These targets were identified based on their molecular weights using the proteomic database for HSV-1 strain 17^{+} [31].

Taken together, our results suggest that the natural products used in this study are endowed with a mechanism of inhibition different from classical anti-HSV-1 agents in clinical use, such as ACV and foscarnet [32]. In addition, the results described in the paragraph above lead us to some possible mechanisms by which compounds **1** and **2** are inhibiting HSV-1 replication, since we observed that the compounds diminished viral proteins belonging to the early (E) phase of HSV-1 replication. We believe that the compounds could be directly acting on this phase or that they might be acting at a previous step of HSV-1 replication, such as the immediate-early (IE) phase. In this last case, considering the role of IE proteins as regulatory molecules [33], we think that the viral target for our natural products would be active even during later events of HSV-1 replication, such as early phase. An alternative explanation for our results might be that compounds **1** and **2** could be targeting, and thus impairing, the formation of the pre-initiation complex, as has been shown for other natural compounds [34]. These possibilities are under investigation in our laboratories through several cellular and molecular approaches.

Finally, we consider that the continuous study on natural compounds may provide future bases for therapeutic molecules endowed with novel mechanisms of action. Along these lines, we propose that the structures of compounds **1** and **2**, brown algae diterpenes obtained from *D. pfaffii* and *D. menstrualis*, respectively, might be encouraging ones for future broad-spectrum antiviral design [15–20].

Affiliations

[1] Laboratório de Estrutura e Regulação de Proteínas, Programa de Pós-Graduação em Química Biológica, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[2] Departamento de Biologia Marinha, Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brasil
[3] Laboratório de Virologia Molecular, Departamento de Biologia Celular e Molecular, Programa de Pós-Graduação em Neuroimunologia, Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brasil
[4] Laboratório de Vírus Respiratórios e do Sarampo, Laboratório de Referência para Influenza e Viroses Exantemáticas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

References

1 *Heldwein EE, Krummenacher C.* Entry of herpesviruses into mammalian cells. Cell Mol Life Sci 2008; 65: 1–16
2 *Roizman B, Sears AE.* Fields virology, 3rd edition. Philadelphia: Lippincott-Raven Publisher; 1996: 2231–2295
3 *Spear PG.* Herpes simplex virus: receptors and ligands for cell entry. Cell Microbiol 2004; 6: 401–410
4 *McKnight JL, Pellett PE, Jenkins FJ, Roizman B.* Characterization and nucleotide sequence of two herpes simplex virus 1 genes whose products modulate alpha-trans-inducing factor-dependent activation of alpha genes. J Virol 1987; 61: 992–1001
5 *Whitley R.* New approaches to the therapy of HSV infections. Herpes 2006; 13: 53–55
6 *Abdel-Haq N, Chearskul P, Al-Tatari H, Asmar B.* New antiviral agents. Indian J Pediatr 2006; 73: 313–321
7 *Waggoner-Fountain LA, Grossman LB.* Herpes simplex virus. Pediatr Rev 2004; 25: 86–93
8 *Lisco A, Vanpouille C, Margolis M.* Coinfecting viruses as determinants of HIV disease. Curr HIV/AIDS Rep 2009; 6: 5–12
9 *De Clercq E.* Antiviral drugs in current use. J Clin Virol 2004; 30: 115–133
10 *Eizuru Y.* Development of new antivirals for herpesviruses. Antiviral Chem Chemother 2003; 14: 299–308
11 *Newman DJ, Cragg GM, Snader KM.* Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003; 66: 1022–1037

12 *Khan MT, Ather A, Thompson KD, Gambari R.* Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral Res 2005; 67: 107–119
13 *Bastow KF.* New acridone inhibitors of human herpes virus replication. Curr Drug Targets Infect Disord 2004; 4: 323–330
14 *Souza TM, Abrantes JL, de A Epifanio R, Leite Fontes CF, Frugulhetti IC.* The alkaloid from 4methylaaptamine isolated from the sponge *Aaptos aaptos* impairs herpes simplex type 1 penetration and immediate-early protein synthesis. Planta Med 2007; 73: 200–205
15 *Barbosa JP, Pereira RC, Abrantes JL, Cirne-Santos CC, Rebello MA, Frugulhetti IC, Teixeira VL. In vitro* antiviral diterpenes from the Brazilian brown alga *Dictyota pfaffii*. Planta Med 2004; 70: 856–860
16 *Barbosa JP, Teixeira VL, Villaça R, Pereira RC, Abrantes JL, Frugulhetti IC.* A dolabellane diterpene from the Brazilian brown alga *Dictyota pfaffii*. Biochem Syst Ecol 2003; 31: 1451–1453
17 *De Souza Pereira H, Leão-Ferreira LR, Moussatche N, Teixeira VL, Cavalcanti DN, Da Costa LJ, Diaz, R, Frugulhetti IC.* Effects of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* on HIV-1 reverse transcriptase. Planta Med 2005; 71: 1019–1024
18 *Pereira HS, Leão-Ferreira LR, Moussatche N, Teixeira VL, Cavalcanti DN, Costa LJ, Diaz R, Frugulhetti IC.* Antiviral activity of diterpenes isolated from the Brazilian marine alga *Dictyota menstrualis* against human immunodeficiency virus type 1 (HIV-1). Antiviral Res 2004; 64: 69–76
19 *Cirne-Santos CC, Teixeira VL, Castello-Branco LR, Frugulhetti IC, Bou-Habib DC.* Inhibition of HIV-1 replication in human primary cells by a dolabellane diterpene isolated from the Brazilian algae *Dictyota pfaffii*. Planta Med 2006; 72: 295–299
20 *Cirne-Santos CC, Souza TM, Teixeira VL, Fontes CF, Rebello M, Castello-Branco LR, Abreu CM, Tanuri A, Frugulhetti IC, Bou-Habib DC.* The dolabellane diterpene dolabelladienetriol is a typical noncompetitive inhibitor of HIV-1 reverse transcriptase enzyme. Antiviral Res 2008; 77: 64–71
21 *Teixeira VL, Cavalcanti DN, Pereira RC.* Chemotaxonomic study of the diterpenes from the brown algae *Dictyota menstrualis*. Biochem Syst Ecol 2001; 29: 313–316
22 *Esquenazi D, Wigg MD, Miranda MM, Rodrigues HM, Tostes JB, Rozental S, Da Silva AJ, Alviano CS.* Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res Microbiol 2002; 153: 647–652
23 *Kuo YC, Chen CC, Tsai WJ, Ho YH.* Regulation of herpes simplex virus type 1 replication in Vero cells by *Psychotria serpens*: relationship to gene expression, DNA replication, and protein synthesis. Antiviral Res 2001; 51: 95–109
24 *Denizot F, Lang R.* Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986; 89: 271–277
25 *De Logu A, Loy G, Pellerano ML, Bonsignore L, Schivo ML.* Inactivation of HSV-1 and HSV-2 and prevention of cell-to-cell virus spread by *Santolina insularis* essential oil. Antiviral Res 2000; 48: 177–185
26 *Gong Y, Matthews B, Cheung D, Tam T, Gadawski I, Leung D, Holan G, Raff J, Sacks S.* Evidence of dual sites of action of dendrimers: SPL-2999 inhibits both virus entry and late stages of herpes simplex virus replication. Antiviral Res 2002; 55: 319–329
27 *Souza TM, De Souza MC, Ferreira VF, Canuto CV, Marques IP, Fontes CF, Frugulhetti IC.* The chloroxoquinolinic derivative 6-chloro-1,4-dihydro-4-oxo-1-(beta-D-ribofuranosyl) quinoline-3-carboxylic acid inhibits HSV-1 adsorption by impairing its adsorption on HVEM. Arch Virol 2007; 152: 1417–1424
28 *De Clercq E.* Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med Res Rev 2000; 20: 323–349
29 *De Ines C, Reina M, Gavin JA, Gonzalez-Coloma A. In vitro* cytotoxicity of norditerpenoid alkaloids. Z Naturforsch 2006; 61: 1–8
30 *Bender FC, Whitbeck JC, Lou H, Cohen GH, Eisenberg RJ.* Herpes simplex virus glycoprotein B binds to cell surfaces independently of heparan sulfate and blocks virus entry. J Virol 2005; 79: 11 588–11 597
31 *Virusys Corporation.* The source of virology related products and services. Available at http://www.virusys.com/HSV-1/HSV-1_ORFS/hsv-1_orfs.html. Accessed March 23, 2009
32 *De Clercq E, Naesens L, De Bolle L, Schols D, Zhang Y, Neyts J.* Antiviral agents active against human herpesvirus HHV-6, HHV-7 and HHV-8. Med Virol 2001; 11: 381–395
33 *Smith RW, Malik P, Clements JB.* The herpes simplex virus ICP27 protein: a multifunctional post-transcriptional regulator of gene expression. Biochem Soc Trans 2005; 33: 499–501
34 *Kuo YC, Lin LC, Tsai WJ, Chou CJ, Kung SH, Ho YH.* Samarangenin B from *Limonium sinense* suppresses herpes simplex virus type 1 replication in Vero cells by regulation of viral macromolecular synthesis. Antimicrob Agents Chemother 2002; 9: 2854–2864