Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
22(4): 881-888, Jul./Aug. 2012

HIV-1 Reverse Transcriptase: a potential target for marine products

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Abstract: HIV-1 reverse transcriptase (HIV-1 RT) is a therapeutic target for the treatment of HIV-positive individuals or those already showing AIDS symptoms. In this perspective, the identification of new inhibitors for this enzyme is of great importance in view of the growing viral resistance to the existing treatments. This resistance has compromised the quality of life of those infected with multidrug-resistant strains, whose treatment options are already limited, putting at risk these individuals lives. The literature has recognized marine organisms and their products as natural sources for the identification of new therapeutic options for different pathologies. In this brief review, we consider the structure of HIV-1 RT and its most common inhibitors, as well as some marine diterpenes originally reported as HIV-1 RT inhibitors to encourage the identification and development of new marine antiviral prototypes.

Article

Received 12 Nov 2011
Accepted 22 Jan 2012
Available online 21 Jun 2012

Keywords:
brown algae
diterpenes
HIV
inhibitor
natural products
reverse transcriptase

ISSN 0102-695X
http://dx.doi.org/10.1590/S0102-695X2012005000084

Introduction

Acquired immune deficiency syndrome (AIDS) is a pandemic disease officially recognized in 1981, which still continues to spread (Girard et al., 2011). So far, AIDS has killed more than 25 million people and. according to the annual survey of the World Health Organization (WHO) in 2010, the number of infected people is stabilizing at around 33.5 million (WHO, 2010). Despite the discovery of antiviral treatment, the epidemic has reached 2.6 million people, of whom 370000 are children under fifteen years (WHO, 2010). This alarming statistic reflects a major problem in global health due not only to the financial aspect but also to the costs in human lives.

AIDS is caused by the Human Immunodeficiency Virus (HIV), a RNA-composed retrovirus of the Retroviridae family (Mulky & Kappes, 2005) (Figure 1) (CDC, 2008; Balzarini, 2004). There are two types of virus, HIV-1 and HIV-2, HIV-2 being the least widespread (e.g., West African countries) with lower rates of mutation, virulence, transmissibility and pathogenicity than HIV-1 (Silva et al., 2008).

HIV infection is characterized by a deep suppression of the immune system of the infected individual due to a progressive depletion of immune cells in the host peripheral blood such as macrophages and, in particular, T lymphocytes. This process makes patients susceptible to opportunistic infections that become fatal due to this immunesuppression (Mosam et al., 2005; De Clercq, 2004).

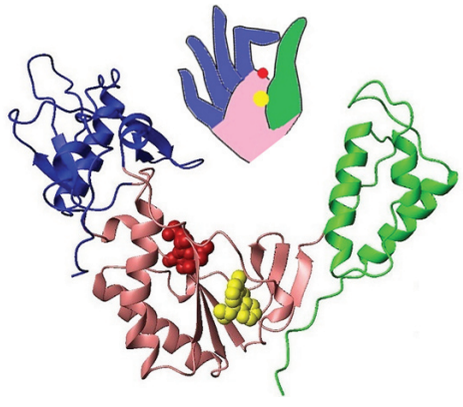


Figure 1. Three-dimensional structure of HIV-1 Reverse Transcriptase. The right-hand conformation representing the fingers subdomain (blue), palm (light red), thumb (green), the active site (red) and a reverse transcriptase inhibitor (yellow) in the binding site.

The Food and Drug Administration (FDA) approved 25 anti-HIV drugs that belong to seven different classes of drugs: nucleoside reverse transcriptase

inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), fusion inhibitors (FI), co-receptor inhibitors (CRI), and integrase inhibitors (INI) (Mehellou & De Clercq, 2010).

HIV-1 has a replicative cycle that depends on different macromolecules, including viral receptors and enzymes (*e.g.*, HIV-Reverse Transcriptase, HIV-Protease, HIV-Integrase). These enzymes are current targets for the antivirals clinically in use, whereas molecular recognition receptors on host CD4 cells (glycoprotein gp120, gp41 linked glycoprotein) are being explored for their potential (Debnath, 2005; Teixeira et al., 2011; Castro et al., 2011; Blanco et al., 2011; Pendri et al., 2011).

HIV-1 Reverse Transcriptase (HIV-1 RT) - still an effective therapeutical target

HIV-1 RT is a heterodimeric enzyme composed of two subunits of 66 kDa and 51 kDa, (p66 and p51, respectively). The p66 subunit (560 amino acids) contains the polymerase and RNase-H active sites, both encoded by the same gene. The p51 subunit contains the first 440 amino acids of p66 and is derived from HIV-1 protease-mediated cleavage of the p66 subunit RNase H domain (Castro et al., 2006; Balzarini, 2004; Singh et al., 2010). The three-dimensional structure of the p66 subunit is compared with a right hand, containing four subdomains: fingers (residues 1-85 and 118-155), palm (86-117 and 156-236), thumb (237-318) and connection (319-426) (Kohlstaedt et al., 1992; Rodgers et al., 1995) (Figure 1).

HIV-1 Reverse Transcriptase catalyzes the synthesis of a double-stranded proviral DNA using the viral genomic RNA. The synthesis of the complementary strand DNA occurs by elongation of the primer tRNA, which is associated with the viral genome. The synthesis of the DNA first strand is initiated from the region of polypurine from the genomic RNA that is resistant to RNase-H and that remains on the new negative strand of DNA (-). All other reverse transcription steps include elongation of the primer DNA (Brautigam & Steitz, 1998; Sarafianos et al., 2009).

HIV-1 RT has the ability to interact with substrates of different conformational structures (double-stranded DNA and single stranded RNA), but with low fidelity or processing capacity. Interestingly, this HIV-1 RT catalytic feature leads to the emergence of mutations at a frequency of about 104 per cycle (Arts & Le Grice, 1998; Patel et al., 1995; Ehteshami & Goette, 2008). Importantly, this high rate of mutation significantly conserves the biological activities of HIV-1 RT, while simultaneously conferring a multidrug-resistant profile to the virus (Das et al., 2004; Martinez-Picado & Martínez, 2008).

The literature describes three main HIV-1 RT inhibitor types, divided by the mechanism of action including: inhibitors of HIV-1 RT polymeric activity; competitive inhibitors subdivided into the class of nucleosides (NRTI) and nucleotide (NtRTI) inhibitors; and non-competitive non-nucleoside inhibitors (NNRTI) (Caffrey 2011, Hatse et al., 1999; Menéndez-Arias, 2002).

Currently, in the most used treatment regimens including *Highly Active Antiretroviral Therapy* (HAART) (Menéndez-Árias et al., 2011), the use of at least one inhibitor of reverse transcriptase is highly recommended, including NRTI such as Zidovudine (AZT), lamivudine, didanosine, zalcitabine, stavudine, abacavir, emtricitabine and Adefovir, or NNRTIs such as delavirdine, Efavirenz and nevirapine (Table 1) (Pretorius et al., 2011). Monotherapy is avoided as well because treatment with only competitive inhibitors slowed the progression of AIDS, but the drug resistance arose quickly (Rao et al., 2004; Martin et al., 2010). Besides the viral resistance issue, these drugs cause different side effects (Table 1) that become extremely toxic in long term use (Temesgen et al., 2006; Sweeney & Klumpp, 2008; Cihlar & Ray, 2010).

Seaweed natural products: a brief presentation of promising molecules

According to Faulkner, the first reported use of marine organisms as a source of chemicals dates to 1600 BC, when the Phoenicians used the secretion of shellfish to produce a dye for cloth (Faulkner, 1992).

Seaweeds are marine organisms that present a great diversity worldwide. In some countries in Asia and Africa where the population daily consumes *Spirulina* (=*Arthrospira*), a blue alga, a low incidence of infection with HIV-1 (AIDS) has been noticed (Ayehunie et al., 1998). These studies also showed that these algae have the property of stimulating the immune response (Teas et al., 2004).

Algae are primarily aquatic organisms that, despite their apparent simplicity present several complex biological systems, including defense, that are found in higher plants (Harper et al., 2001;Vidotti & Rollemberg, 2004). These biological pathways involve different molecules that can be promising for treating certain pathologies, including HIV-infection (Alakurtti et al., 2006; Vo & Kim, 2010; Kim & Karadeniz, 2011).

The marine environment provides a rich source of chemical diversity for the screening and identification of new compounds with desirable antiviral properties. The use of marine natural products as anti-HIV agents has been described in the literature, promising molecules including the phlorotannins from brown algae (Phaeophyceae), sulfated polysaccharides from

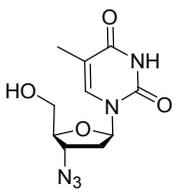
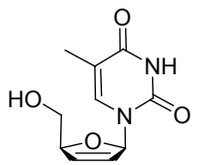
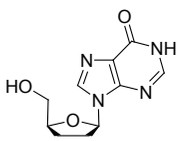
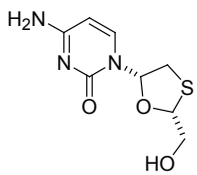
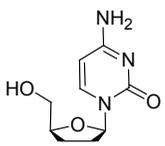
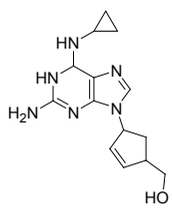
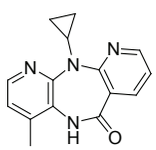
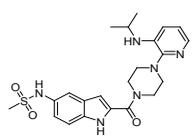
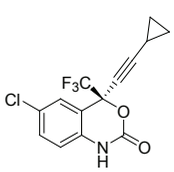
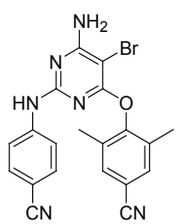
Chlorophyceae (green algae), Rhodophyceae (red algae) and Phaeophyceae (brown algae) and lectins such as Griffithsin from *Griffithsia* sp. (red algae) (Alakurtti et al., 2006; Vo & Kim, 2010; Kim & Karadeniz, 2011).

Terpenes as NNRTI models: looking at future anti-HIV treatment options?

NNRTI usually manifest side effects milder than those resulting from treatment with nucleosides since they are not analogues of natural compounds and involve no host cell biochemical machinery. Although the therapeutic potential of NNRTI has been compromised by the rapid development of resistance, they have been useful in combination therapy with nucleoside RT and protease inhibitors (Barreca et al, 2004). Thus, research exploring novel classes of safe and effective agents with low risk of cross-resistance with other antiretroviral drugs is currently in urgent need (WHO, 2011).

Many marine organisms are major producers of secondary metabolites derived from isoprene units, including the monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40). Among them, seaweeds are one of the main producers of these substances (Blunt et al., 2006). Of the 15,000 secondary metabolites of marine origin, approximately 55% are derived from terpenoids. This proportion can be much higher in some phyla, representing up to 90% of the metabolites isolated.

Table 1. Side effects of the most used NRTI (up) and NNRTI (below) of HIV-1 Reverse Transcriptase.

Antiviral	Structure	Collateral/ side effects	Antiviral	Structure	Collateral/ side effects
Zidovud ine (AZT)		Myelosuppression with neutropenia and anemia, Nausea and vomiting. Asthenia, malaise, headache, insomnia, Skin hyperpigmentation, nail and mucous membranes. Rare: lactic acidosis with hepatic steatosis (fatal if severe).	Stavudine (d4T)		Peripheral neuropathy, pancreatitis, asymptomatic acidemia, lipoatrophy. Rare: lactic acidosis with hepatic steatosis (fatal if severe).
Didanosine (ddI)		Gastrointestinal intolerance (nausea and diarrhea), peripheral neuropathy, pancreatitis, asymptomatic acidemia, lipoatrophy. Rare: lactic acidosis with hepatic steatosis (fatal if severe).	Lamivudine (3TC)		lactic acidosis with hepatic steatosis
Zalcitabine (ddC)		Peripheral neuropathy, stomatitis, esophageal ulcerations. Rare acidosis lactate with hepatic steatosis (fatal if severe).	Abacavir (ABC)		Reaction of systemic respiratory hypersensitivity and / or gastrointestinal, usually with fever and without mucosal involvement.
Nevirapine		Rash, Stevens-Johnson Syndrome, Elevated transaminases blood level, hepatitis, severe hypersensitivity reaction.	Delavirdine		Rash, headache, elevation of transaminases
Efavirenz		Rash, Stevens-Johnson Syndrome. Neuropsychiatric symptoms: sleep disturbances (restless sleep, insomnia, drowsiness, nightmares, bizarre dreams), dizziness, vertigo, irritability, agitation, depression, euphoria, difficulty concentrating, amnesia, hallucinations. Elevation of transaminases. Dyslipidemia.	Etravirine		Rash, Stevens-Johnson syndrome, toxic epidermal necrosis and multiform erythema, as well as hypersensitivity reactions, hepatic failure

Marine algae are among the main producers of diterpenes showing antibacterial, antiviral, antifungal, and other biological activities (Harper et al., 2001). The literature describes diterpenes isolated from the *Dictyota* alga species with potential antiviral activity (DePaula et al., 2011). The main diterpene compounds isolated from *Dictyota menstrualis* (Hoyt) Schnetter, Hörning & Weber-Peukert, identified as 6-hydroxydichotoma-3,14-dien-1,17-dial (**1**) and its acetate derivative 6-acetoxydichotoma-3,14-dieno-1,17-dial (**2**) (Figure 2), exhibit inhibitory activities against HIV-1 replication affecting HIV-1 reverse transcriptase (RT) activity in a dose-dependent form (Pereira et al. 2004, 2005). A similar antiviral profile was observed in a dollabelane diterpene isolated from *Dictyota pfaffii* Schnetter (**3**-**5**) (Figure 2). Apparently these marine products act as NNRTI.

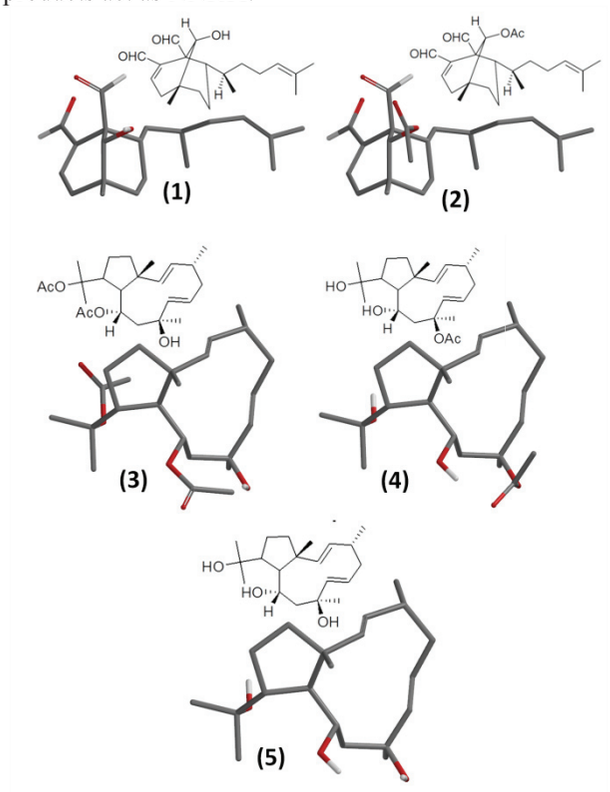


Figure 2. Antiviral diterpenes isolated from the brown algae *D. menstrualis* (1-2) and *D. pfaffii* (3-5).

The NNRTI were first described when TIBO and nevirapine derivatives were discovered during research on HIV-1 RT inhibition (Pauwels et al., 1990, Shih et al., 1991). NNRTI are chemically diverse, with one class being considerably different from another in terms of chemical composition and size. This is analogous to the complexity of marine natural products, with a great variety of structures with different degrees of biological activity (De Clercq, 1998). NNRTI have in common the affinity for the extremely flexible hydrophobic p66 chain located near the active site (approximately 10 Å away) and located between the β-sheet-6-9-β-β and β-10-12-13-β-β-14 from the palm domain, called the "non-nucleoside inhibitor binding pocket" (NNIBP) (Boyer et al., 1994a, 1994b; Sluis-Cremer et al., 2004; Martin et al., 2010; Zhan et al., 2011). The inhibition mechanism is due to the expansion of the region of NNBIP, since this hydrophobic "pocket" is closed during the active period of TR. The opening of this region involves a large displacement of the aromatic side chains of Tyr181, Tyr188 and Trp229 and a rotation of the leaves β-β-12-13-β-14, resulting in a breakdown of the primer grip in the direction that the complex primer-template moves during the subsequent incorporation of nucleotides (Das et al., 2004) (Figure 3).

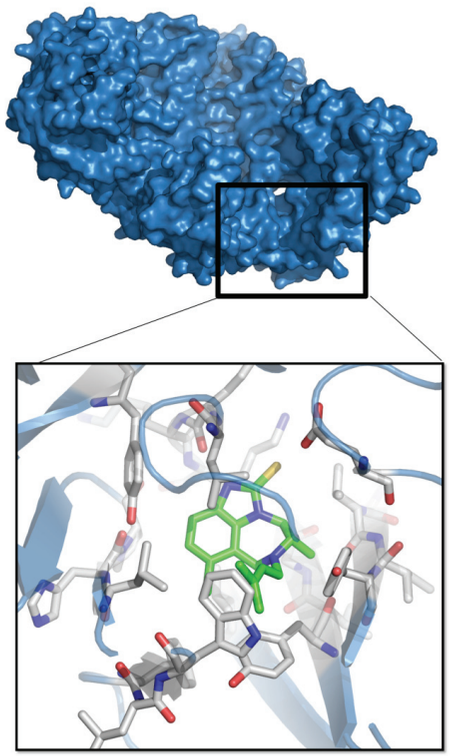


Figure 3. Three-dimensional structure of the NNRTI binding site. In green, the inhibitor TIBO.

Our group showed that the diterpene 8,10,18-trihydroxy-2,6-dolabelladiene (THD, 5), obtained from the extract of *Dictyota pfaffi* or by reducing 10,18-diacetoxy-8-hydroxy-2,6-dolabelladiene (**3**), showed significant antiviral activity, up to 3 times higher with this chemical modification (Barbosa et al., 2003; Barbosa et al., 2004; Cirne-Santos et al., 2006). Our experimental study confirmed a dose-dependent anti-HIV-1 TR activity, with an IC50 of 16.5 μM, inhibition levels ranging from 27% (3 μM) to 95% (100 μM), and 85% viability of peripheral blood mononuclear cells (PBMC) at concentrations of 200 μM (Barbosa et al. 2003; Cirne-Santos et al., 2006).

Importantly, dolabelladienetriol blocked

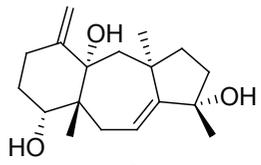
the synthesis and integration of HIV-1 provirus and completely abrogated viral replication in primary cells. Studies of the kinetic mode of action revealed that dolabelladienetriol is a nonnucleoside RT inhibitor (NNRTI), acting as a noncompetitive inhibitor, with a Ki value equal to 7.2 μM.

Interestingly, dolabelladienetriol provided an additive effect with the nucleoside RT inhibitor AZT, and a synergistic effect with the protease inhibitor atazanavir sulphate. There was no increment of the anti-HIV-1 effect resulting from the combination between dolabelladienetriol and the NNRTI nevirapine. Using a large panel of HIV-1 isolates harboring NNRTI resistance mutations, we found no cross-resistance between dolabelladienetriol and clinically available NNRTIs (Cirne-Santos et al., 2008).

Our group also described two other diterpene skeleton dichotomanes with anti-HIV-1 RT antiviral action, (6R)-6-hydroxydichotoma-3,14-diene-1,17-dial (HDD, **1**) and its acetate derivative (6R)-6-acetoxydichotoma-3,14-diene-1,17-dial (DDA, **2**) extracted from the brown algae *Dictyota menstrualis* (Pereira et al., 2004; Pereira et al., 2005). While HDD showed a value of inhibition of HIV-RT of IC50 10 μM, the value for DDA was 35 μM (Pereira et al., 2004; Pereira et al., 2005). None of these diterpenes affected the DNA-dependent DNA-polymerase (DDDP) activity of HIV-1 RT. The RNA-DDP activities of AMV-RT and MMLV-RT enzymes were also inhibited by HDD and DDA. In contrast to the HIV-1 enzyme, the DDDP activities of AMV-RT and MMLV-RT enzymes were significantly reduced. Taken together, our results demonstrate that HDD is a more effective inhibitor of the viral reverse transcriptases from HIV-1, AMV and MMLV than DDA.

The kinetic analyses of the HIV-1 RT demonstrate that both diterpenes have similar mechanisms of inhibition of RDDP activity (Pereira et al., 2005). The mechanism of inhibition of HIV-RT by terpenes was evaluated and apparently occurs by forming a "butterfly-like" structure, as observed for NNRTIs inhibitors (Castro et al., 2006, De Clercq, 2004).

More recently, four diterpenes from other Dictyotaceae, *Canistrocarpus cervicornis* (Kützing), three dolastanes and a secodolastane diterpene were also described with anti HSV-1 (Vallim et al. 2010) and anti-HIV-1-RT profiles including (4*R*,9*R*,14*S*)-4,9,14-trihydroxy-dolast-1(15),7-diene (**6**), the isolinearol (**7**), (4*R*,7*R*,14*S*)-4,7,14-trihydroxydolast-1(15),8-diene (**8**) and (4*R*,7*R*,14*S*)-4α ,7-diacetoxy-14-hydroxydolast-1(15),8-diene (**9**). Although these natural products were known since the 80's from *Dictyota cervicornis* (Teixeira et al, 1986a, 1986b; Kelecom & Teixeira 1988), the antiviral activity was only identified in 2010. The results of anti-HIV-1 RT led to the registration of a patent application in 2010 (Paixão et al., 2010).



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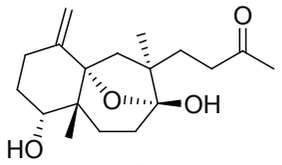

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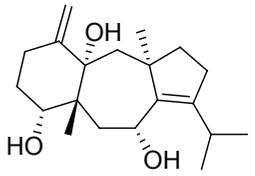

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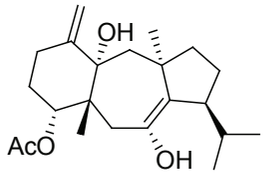

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Conclusion

Far from pointing out terpenes as the only marine products able to inhibit HIV-RT, this brief review reinforces the biotechnological potential and the need to explore marine resources as thoroughly as reasonably possible in order to find new treatments not only for HIV, but also for other infectious diseases such as herpes.

Acknowledgment

The authors thank CNPq-Universal, CAPES, FOPESQ-UFF and FAPERJ for financial support and fellowships.

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