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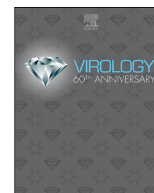

Characterization of designed, synthetically accessible bryostatin analog HIV latency reversing agents

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ABSTRACT

HIV latency in resting CD4+ T cell represents a key barrier preventing cure of the infection with antiretroviral drugs alone. Latency reversing agents (LRAs) can activate HIV expression in latently infected cells, potentially leading to their elimination through virus-mediated cytopathic effects, host immune responses, and/or therapeutic strategies targeting cells actively expressing virus. We have recently described several structurally simplified analogs of the PKC modulator LRA bryostatin (termed bryologs) designed to improve synthetic accessibility, tolerability in vivo, and efficacy in inducing HIV latency reversal. Here we report the comparative performance of lead bryologs, including their effects in reducing cell surface expression of HIV entry receptors, inducing proinflammatory cytokines, inhibiting short-term HIV replication, and synergizing with histone deacetylase inhibitors to reverse HIV latency. These data provide unique insights into structure-function relationships between A- and B-ring bryolog modifications and activities in primary cells, and suggest that bryologs represent promising leads for preclinical advancement.

1. Introduction

Infection with human immunodeficiency virus (HIV) almost invariably causes severe damage to the host immune system, leading to the development of acquired immunodeficiency syndrome (AIDS). This process can be inhibited by the administration of antiretroviral therapy (ART), which blocks HIV replication and often reduces HIV RNA viral loads to levels below the limit of detection using standard clinical assays (approximately 50 copies per mL of plasma) (Dinoso et al., 2009; Gulick et al., 1997; Perelson et al., 1997). However, if ART is stopped, viral replication rapidly resumes allowing disease progression to continue (Chun et al., 1999a). As a result, ART requires strict and life-long compliance to maintain suppression. While multiple factors might contribute to the persistence of HIV during ART, one important source of replication-competent HIV in treated patients is latently-infected CD4 + T cells (Chun et al., 1995; Finzi et al., 1999; Finzi et al., 1997; Wong et al., 1997). These long-lived latently-infected cells harbor integrated proviruses that express little or no viral RNA and no viral proteins, but episodically produce infectious virions upon appropriate stimulation of the host cell (Chun et al., 1997; Finzi et al., 1997). Elimination of these reservoir cells, the chronic source of re-infection, is therefore critical for HIV eradication from infected individuals.

One approach for purging latent reservoir cells is to induce expression of the latent provirus, thereby rendering the host cell susceptible to viral cytopathic effects, immune effector mechanisms, and/or therapeutic approaches targeted towards viral proteins (Reviewed in (Marsden and Zack, 2009, 2010; Marsden and Zack, 2015)). Several clinical efforts to induce HIV expression in latently infected cells have been undertaken, including administration of interleukin 2 (IL-2) either alone (Chun et al., 1999b) or in combination with anti-CD3 antibodies (OKT3) (Kulkosky et al., 2002; Prins et al., 1999; van Praag et al., 2001). Histone deacetylase inhibitors (HDACi) (Archin et al., 2012; Lehrman et al., 2005; Rasmussen et al., 2014) and the anti-alcoholic-abuse agent disulfiram (Elliott et al., 2015) have been tested in a similar manner. More recent work in this area includes a combined approach using Vacc-4 ×, recombinant human granulocyte macrophage colony

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stimulating factor vaccination, and HDACi romidepsin, which was tested in a phase 1B/2A trial (Leth et al., 2016). The treatments described in these important pioneering studies had varying effects on HIV expression and the relative frequency of latently infected cells, with several capable of increasing HIV RNA expression or inducing limited reduction of the latent reservoir in a subset of patients. However, none of them entirely eliminated replication-competent HIV from the infected individuals. The more aggressive therapies involving OKT3 and/or IL-2 also resulted in toxic side-effects due to generalized immune activation (Prins et al., 1999; van Praag et al., 2001). This issue of toxicity associated with HIV LRAs is challenging because expression of HIV proviral DNA is connected to the activation state of the host cell, prompting concerns that the most effective HIV latency reversal agents might also cause global T cell activation, resulting in hypercytokinemia or a "cytokine storm" that could create unacceptable risks and thus preclude their clinical use. Therefore, while the "kick and kill" approach for eliminating latent HIV has been explored in proof-of-concept clinical studies, more effective and better tolerated agents are required if latency purging strategies are to be fully effective.

Protein kinase C (PKC) modulators, including prostratin, ingenol, and bryostatin are of interest in the context of HIV eradication efforts because they can activate the transcription factor NF-kappa B (Williams et al., 2004) and induce HIV from latency in various cell and animal models, as well as primary cells from ART-treated patients (Baxter et al., 2016; Beans et al., 2013; Bullen et al., 2014; Darcis et al., 2015; DeChristopher et al., 2012; Jiang et al., 2015; Kinter et al., 1990; Marsden et al., 2017; Perez et al., 2010; Qatsha et al., 1993). Indeed, the first phase I clinical trial of bryostatin 1 (hereafter referred to as bryostatin) has been conducted in ART-treated, HIV-infected individuals. This study found bryostatin to be safe at the 10 and 20 μg/m^2 doses tested, with the results indicating that higher doses would likely be needed to induce the desired effects on PKC activity in vivo (Gutierrez et al., 2016). Higher doses (up to approximately 50 μg/m^2) are tolerated, as demonstrated in several oncology trials with bryostatin, but the identification of analogs with an expanded therapeutic window is a significant objective of ongoing research.

Despite bryostatin's clinical promise, this lead compound is isolated in very low and variable yields from its marine source (14 t of *Bugula neritina* yielded only 18 g of Good Manufacturing Practice-grade [GMP] bryostatin), raising cost and environmental concerns about its sustainable supply from natural sources (Schaufelberger et al., 1991). To address this problem, aquaculture was tried but abandoned (Mendola, 2003). Synthetic biological approaches remain in early stages due to complications arising from cultivation of the symbiotic bacterium that produces bryostatin (Miller et al., 2016; Trindade-Silva et al., 2010). The current supply of GMP bryostatin produced in the 1990s is nearly depleted. Thus while bryostatin continues to serve as a significant therapeutic lead and an important clinical candidate for multiple indications, the aforementioned therapeutic window and supply issues have hampered its advancement. Recognizing that bryostatin, like many natural products, is neither evolved nor optimized for the treatment of human disease, we previously reported the first analogs of bryostatin that are more synthetically accessible and exhibit activities comparable or better than bryostatin (Wender et al., 2014, 2015). More recently we reported a scalable total synthesis of bryostatin that addresses the clinical supply problem, and importantly is readily adapted to enable the design and synthesis of superior analog compounds, lead examples of which are evaluated herein (Wender et al., 2017).

To address these issues of supply and sub-optimal activity, we previously reported a function-oriented approach to designed synthetically-accessible bryostatin analogs (DeChristopher et al., 2012; Wender et al., 1988). We demonstrated that we could effectively recapitulate the PKC affinities and activities of the natural product with bryostatin analogs or "bryologs" featuring structural variations in the A- and B-rings of the macrocyclic scaffold (Fig. 1). This approach also provides the opportunity to tune the activity of analog compounds to optimize

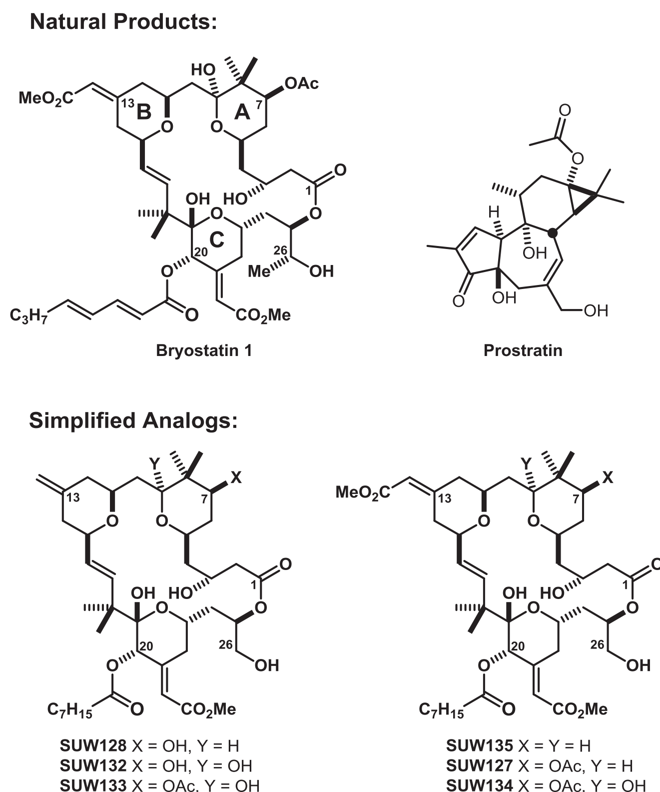

Fig. 1. Structures of compounds. Chemical structures of bryostatin 1, prostratin, and simplified bryostatin analogs (bryologs) are shown. Analogs SUW128, SUW133, SUW135, SUW127, and SUW134 here correspond to previously published (DeChristopher et al., 2012) analogs 2, 4, 5, 6, and 7 respectively. SUW132 corresponds to compound 4.55 (Schrier, 2011), and was prepared in an analogous manner to SUW128 and SUW133 (Wender et al., 2014).

their performance via targeted structural manipulation of the bryostatin scaffold. Significantly, these designed bryologs potently induce HIV expression in a J-Lat model for HIV latency (DeChristopher et al., 2012). We further established that one particularly efficacious bryolog (SUW133) could induce HIV from latency ex vivo from CD4 + T cells derived from HIV-infected ART-treated patients and in vivo in humanized BLT mice (Marsden et al., 2017).

In the current study, we address the activities of these bryologs as required for their preclinical advancement with a focus on their comparative ability to alter HIV entry receptor levels and induce proinflammatory cytokines in primary peripheral blood mononuclear cells (PBMC), affect HIV spread in activated CD4 + T cells, and synergize with HDACi to reverse HIV latency. These experiments provide important insights into how structural variations of bryologs affect HIV latency reactivation and influence subsequent viral spread. Significantly, we have found that improved HIV latency reversal activity can be achieved with lead bryologs without a corresponding increase in induction of potentially damaging cytokines.

2. Results

2.1. Bryologs reduce cell surface HIV entry receptor levels

Structures for the synthetic bryologs, bryostatin and prostatin used in this study are provided (Fig. 1). We first explored whether the novel bryologs share potentially beneficial activities with the parent compound bryostatin, beyond their ability to induce HIV from latency. Not unlike bryostatin and prostratin (a non-tumor promoting phorbol ester that similarly activates latent HIV by activating protein kinase C), the

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