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Short communication

Novel diversity-oriented synthesis-derived respiratory syncytial virus inhibitors identified via a high throughput replicon-based screen

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ABSTRACT

Respiratory syncytial virus (RSV) infections affect millions of children and adults every year. Despite the significant disease burden, there are currently no safe and effective vaccines or therapeutics. We employed a replicon-based high throughput screen combined with live-virus triaging assays to identify three novel diversity-oriented synthesis-derived scaffolds with activity against RSV. One of these small molecules is shown to target the RSV polymerase (L protein) to inhibit viral replication and transcription; the mechanisms of action of the other small molecules are currently unknown. The compounds described herein may provide attractive inhibitors for lead optimization campaigns.

Respiratory syncytial virus (RSV) is an enveloped, negative sense RNA virus that is a leading cause of acute respiratory infections in young children, the elderly, and immunosuppressed individuals. In the United States, recent studies estimate that approximately 2 million children under the age of 5 require medical attention annually due to RSV infection (Hall et al., 2009), with young infants at the greatest risk of hospitalization (Hall et al., 2013). Globally, RSV is thought to cause disease in approximately 30 million young children per year, with developing nations bearing the greatest burden of mortality (Nair et al., 2010). RSV infection is also a major cause of respiratory infections in adults, with more severe disease in the elderly; approximately 17,000 adults die annually in the United States as a result of RSV infection [reviewed in (Collins and Melero, 2011)]. Despite the significant disease burden, safe and effective vaccines and therapeutics are not currently available, emphasizing the need for new treatments (Collins and Melero, 2011; Simoes et al., 2015).

We recently described the development of a subgenomic replicon-based high throughput screen to identify new inhibitors of RSV (Laganas et al., 2015; Plant et al., 2015; Tiong-Yip et al., 2014). This replicon consists of the RSV genome from which the small hydrophobic (SH), glycoprotein (G) and fusion (F) genes were deleted and replaced with an antibiotic resistance selection marker (Malykhina et al., 2011). As the replicon still expresses all the proteins required for mRNA transcription and genome replication, the large polymerase subunit (L) protein, the phosphoprotein (P), the transcription elongation factor M2-1, and the nucleoprotein (N), it can be maintained in cell culture. Here we report the use of this replicon system for identification of three novel diversity-oriented synthesis-derived scaffolds, represented by BRD9101, BRD4517 and BRD3482 (Fig. 1), which inhibit the replication of RSV.

A high throughput screen of the Broad Institute's diversity-oriented synthesis (DOS) compound collection was performed with cryopreserved APC126-E cells (Tiong-Yip et al., 2014) at a

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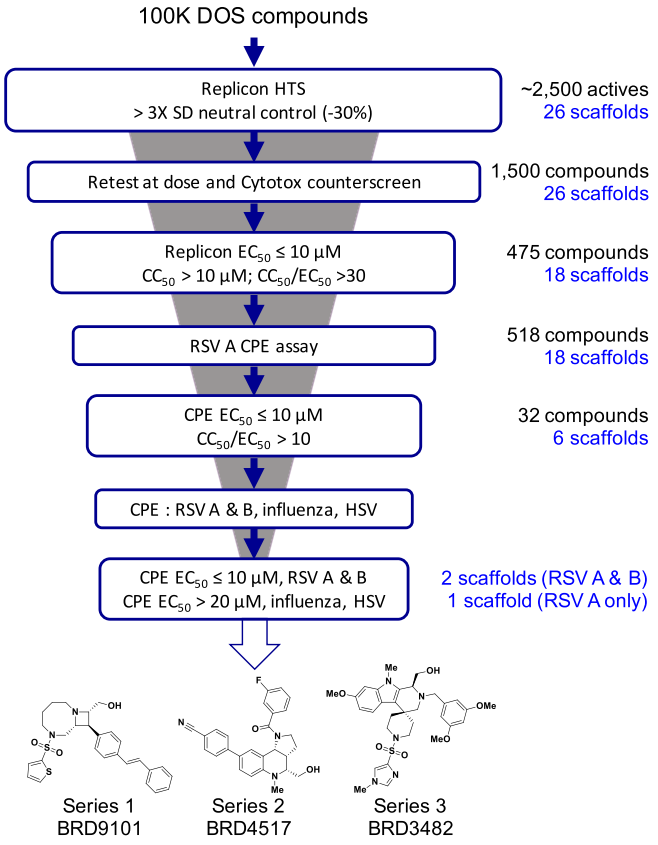
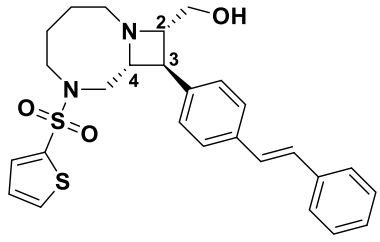


Fig. 1. Screening cascade to identify DOS inhibitors of RSV replication. The screening cascade comprised of a primary RSV replicon screen, with downstream cytotoxicity counter-screens and additional live virus assays to identify compounds of interest. Live virus assays included RSV, influenza, and HSV CPE assays. Three final scaffolds of interest were identified, two of which were active against both RSV A and B strains. The number of compounds and scaffolds progressed at each stage are indicated on the right.



Stereochemistry (C2,C3,C4)
RSV inhibition at 10 µM

S,S,R 68%	S,S,S -	R,S,R 20%	R,S,S <5%
S,R,R <5%	S,R,S 8%	R,R,R -	R,R,S <5%

Fig. 2. Series 1 compounds show stereoselective inhibition of the RSV replicon. HTS data from the RSV replicon data are shown for all available stereoisomers of a representative compound from Series 1 (BRD9101). The stereochemistry of C2, C3, and C4 is indicated, along with the % inhibition of the RSV replicon; the S,S,R stereoisomer was the most potent, while the R,S,R diastereomer, differing only in the extracyclic hydroxyl group, showed more modest inhibition. All other tested diastereomers were inactive.

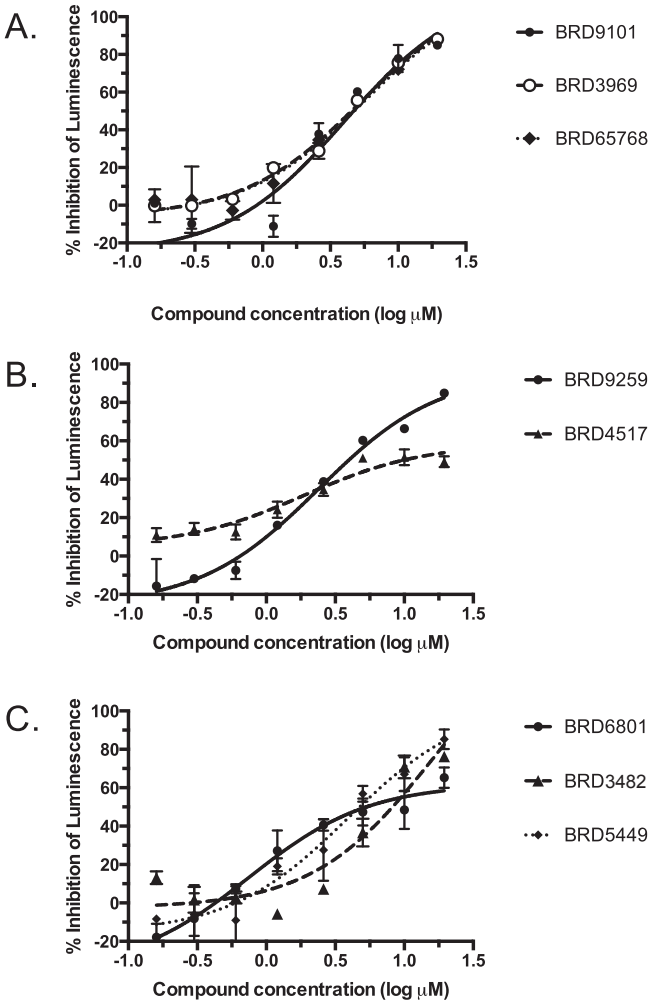


Fig. 3. Hit compounds inhibit RSV replicon expression. APC126-E cells expressing a subgenomic RSV replicon with a luciferase gene were treated with ascending doses of hit compounds for 48 h and then assessed for total cellular luminescent signal. The selected compounds showed dose-dependent inhibition of luciferase suggesting inhibition of either replication or expression of the replicon system. Panels A, B, and C show compounds from Series 1, 2, and 3, respectively. EC_{50} values are provided in Table 1.

single drug concentration of 10 µM, resulting in approximately 2500 initial hits that exhibited >30% inhibition of the replicon (hit rate = 2.5%). Fig. 1 delineates the screening cascade that was used to identify validated hits. Based on cheminformatic analyses of the high throughput data using the Broad's internal SAR analysis tools (Mulrooney et al., 2013), hits showing good activity, structurally attractive features or preliminary SAR were prioritized. For example, BRD9101 showed good inhibition within the primary screen (70% inhibition at 10 µM). Of the six stereoisomers tested, only one additional stereoisomer showed any level of inhibition within the primary screen (Fig. 2). Analysis of closely related analogs identified BRD3969 and BRD65768 as having weak but significant activity against RSV, while also offering structural alternatives to the less attractive thiophene and styrene motifs (Supporting Fig. 1). Additional SAR gathered from the primary screen pointed to the importance of the heteroaromatic sulfonamide off of the diazocane ring, as other sulfonamides did not show activity (Supporting Fig. 1). BRD9101, BRD3969 and BRD65768 were included with 1500 compounds that were cherry-picked for retest at dose in the replicon and cytotoxicity assays. Compounds that