Development of Diheteroamide-based Small Molecules as a Novel Class of HIV-1 Inhibitors with Improved Safety Profile

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Background

Initial screens of compounds capable of inhibiting HIV-1 gene expression identified a stilbenebased compound 5350150 (Ref. 1) which significantly reduced the production of HIV-1 Gag and Env. However, the known photolabile/toxic properties of stilbene compounds rendered the molecule unsuitable for long term treatment option. Here we report the identification of GPS 488, a noncytotoxic analogue of 5350150 where the C=C double bond has been replaced by an amide isostere, that GPS 488 markedly reduced associated cytotoxicity, and display anti-HIV activity against a comprehensive panel of multidrug-resistant strains.

Methods

An exploratory library of fifty 5350150-amide analogues was prepared using the acyl fluoride–TMS amine condensation protocol. The evaluation of the antiretroviral activity of this collection was determined by a T-cell reporter assay that expresses green fluorescent protein upon HIV infection. The level of infection was monitored using flow cytometric analysis (guavaSoft 2.2™ software, Guava HT8, Millipore™). The antiviral activity of compound GPS 488 was evaluated in the assay that measure inhibition of HIV-1 spread in a co-culture of CEM-GXR cells containing one percent of HIV-1 infected cells. Infection was performed in a 96-well plate containing serial dilutions of molecule GPS 488, and the half maximal effective concentration (EC50) values were determined on day 3 post infection. To investigate the potential adverse effect on cell viability, we employed Guava ViaCount Assay (Millipore) on the same reporter cells. In brief, CEM-GXR cells were seeded in 96-well plates 80,000 cells/well in the absence or presence of compound GPS 488 in concentrations ranging between 1.25µM and 100µM. Twenty-four hours later, 25 µL of cell suspension was mixed with 225 µL of Guava ViaCount™ reagent, and the mixture was incubated for 5 min at room temperature. Sample acquisition and data analysis were performed with the selection of EasyFit analysis feature using the ViaCount™ software module.

Results

Anti-HIV-1 activity was found to varying degrees of the 5350150 amide analogues, and one of the most potent compounds GPS488, was studied in detail. Compound GPS488 exhibited activity against wild-type HIV-1NL4.3 (subtype-B), and HIV-1 97USSN54 (subtype-A) with EC50 values of 1.3µM and 1.0µM, respectively. For multidrug-resistant viruses, GPS488 inhibited an HIV-1 variant resistant to both NRTI and NNRTI with EC50 of 1.2µM. Compound GPS488 was also active against viruses resistant to protease inhibitors, integrase inhibitors, and CCR5 antagonist inhibitors with EC50's of 1.2µM, 1.0µM, and 1.0µM, respectively. The potential cytotoxicity of GPS488 was measured by Guava ViaCount Assay. At the range of concentrations tested, a decrease in cell viability from 98.9% at 1.25µM to 59.2% at 100µM was observed.

Table 1. Mutations in HIV-1 proteins associated with resistance to ARVs

Drug resistant HIV-1 Strain	Viral protein	Primary Mutations	EC50 for GPS 488	Resistance to ARVs
E00443	Reverse - Transcriptase	K103N, D67N, K70R, Y115F, Q151M, M184V, K219Q	1.2µM	EFV, NVP, 3TC, ABC, AZT, and FTC
2948	Protease	G48V, L90M	1.2µM	ATV and LPV
11845	Integrase	G140S, Q148H	1.0µM	RAL, EVG, and DTG
MVC-res	Envelope-V3	A19T, L20F, T22A, E25D, I26V.	1.0µM	MVC

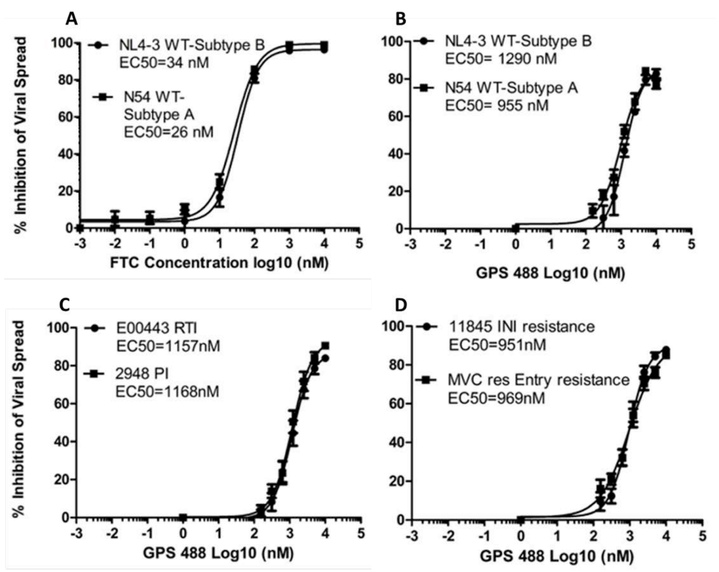

Figure 1. Anti-HIV-1 activity of GPS 488 against wild-type subtype A and B, and drug-resistant viruses. A: Emtricitabine (FTC) against wild-type HIV-1NL4-3 (subtype B), and 97USSN54 (subtype A) as a positive control. B: GPS 488 against wild-type subtype A and B. C: GPS 488 against (N)NRTI-resistant isolate, and PI-resistant isolate. D: GPS 488 against INI-resistant isolate, and maraviroc-resistant R5 strain.

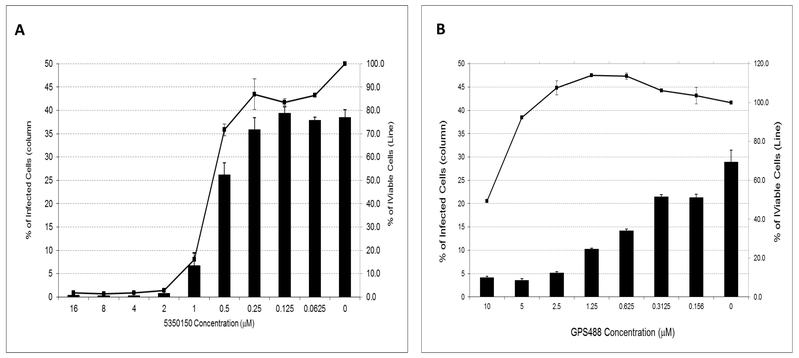

Figure 2. Evaluation of anti-HIV-1BaL activity (column) and GXR-CEM cell viability (line) for 5350150 (A) and GPS 488 (B) at concentrations between 62.5 nM and 16 µM for 5350150, and between 156nM and 10 µM for GPS 488. HIV-1 infection and viability in CEM-GXR cells was assessed by measuring GFP positive cells and cell count, respectively 3 days after infection. For viable cell counts, the gate in a flow cytometer was set to cover 95% of the freshly passaged uninfected CEM-GXR cell. Results were expressed from three independent experiments for both the anti-HIV-1 activity and cell viability assays

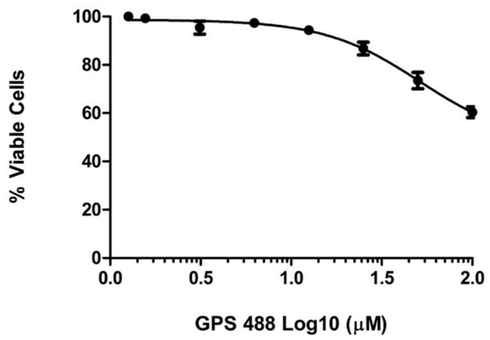

Figure 3. Evaluation of GPS 488 on CEM-GXR cell viability in the Guava ViaCount assay. Cells were analyzed after 24 h incubation with GPS 488 in concentrations ranging between 1.25 µM and 100 µM. Results are expressed as the percentage (%) of viable cells ± SEM of three independent experiments.

Conclusions

- Compound GPS 488 inhibits both HIV-1 subtype A, R5 tropic and subtype B, X4 tropic at the low micromolar range.
- Compound GPS 488 remains active against a panel of HIV-1 strains that are resistance to the current 4 major drug targets at the EC50 values between 1.0 µM and 1.2 µM.
- A decrease in cell viability from 98.9% to 59.2 % was observed in the presence of 100 µM of GPS 488.
- In comparison with stilbenebased compound 5350150, compound GPS 488 markedly reduced associated cytotoxicity in CEM-GXR cells.

Ref. 1. Wong RW et al. Characterization of novel inhibitors of HIV-1 replication that function via alteration of viral RNA processing and rev function. *Nucleic Acids Research* 2013, 41:9471-9483.

Conflict of Interest Disclosure: "I have no conflicts of interest"