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Novel structurally related compounds reactivate latent HIV-1 in a *bcl-2*-transduced primary CD4+ T cell model without inducing global T cell activation

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Background: The latent reservoir of HIV-1 in resting memory CD4+ T cells is a major barrier to curing HIV-1 infection. Eradication strategies involve reactivation of this latent reservoir; however, agents that reactivate latent HIV-1 through non-specific T cell activation are toxic.

Methods: Using latently infected Bcl-2-transduced primary CD4+ T cells, we screened the MicroSource Spectrum library for compounds that reactivate latent HIV-1 without global T cell activation. Based on the structures of the initial hits, we assembled ~50 derivatives from commercial sources and mostly by synthesis. The dose–response relationships of these derivatives were established in a primary cell model. Activities were confirmed with another model of latency (J-Lat). Cellular toxicity and cytokine secretion were tested using freshly isolated human CD4+ T cells.

Results: We identified two classes of quinolines that reactivate latent HIV-1. Class I compounds are the Mannich adducts of 5-chloroquinolin-8-ol. Class II compounds are quinolin-8-yl carbamates. Most EC_{50} values were in the 0.5–10 μM range. HIV-1 reactivation ranged from 25% to 70% for anti-CD3+ anti-CD28 co-stimulation. All quinolin-8-ol derivatives that reactivate latent HIV-1 follow Lipinski's Rule of Five, and most follow the stricter rule of three for leads. After 48 h of treatment, none of the analogues induced detectable cytokine secretion in primary resting CD4+ T cells.

Conclusions: We discovered a group of quinolin-8-ol derivatives that can induce latent HIV-1 in a primary cell model without causing global T cell activation. This work expands the number of latency-reversing agents and provides new possible scaffolds for further drug development research.

Keywords: virus, latency, reactivation, primary cell model, quinolin-8-ol

Introduction

Highly active antiretroviral therapy (HAART) can successfully reduce plasma HIV-1 levels in infected individuals to below the detection limit of clinical assays (<50 copies/mL) and reverse disease progression.[1] However, the latent reservoir in resting memory CD4+ T cells remains a major barrier to virus eradication. In these latently infected cells, integrated provirus remains transcriptionally silent as long as the host cell remains in a resting state.[2] The absence of viral proteins allows evasion from immune surveillance, and HAART, which only targets replicating virus, cannot eradicate latent HIV-1.[3] However, following cellular activation, these latent HIV-1 genomes can be transcribed and virus will be produced, leading to quick rebound of viraemia upon the discontinuation of HAART. The extreme stability of this latent reservoir makes life-long HAART necessary. Given the potential for resistance, which might result in HAART failure, and the toxicity and expense of life-long HAART, elimination of the latent reservoir is an important goal.

A widely discussed approach to eliminating this reservoir involves reactivating latent HIV-1. Presumably cells harbouring latent HIV-1 will die upon reactivation, due to viral cytopathic effects or host cytolytic mechanisms. Even if this assumption is not correct and additional strategies that kill productively infected cells are needed, reactivation of HIV-1 gene expression is the important first step.

Using different cell lines and primary cell models, several groups have identified agents that reactivate latent HIV-1, including the histone deacetylase (HDAC) inhibitors suberoylanilide hydroxamic acid (SAHA),[4] Trichostatin A,[5] valproic acid,[6] and protein kinase C (PKC) activators prostratin[7] and bryostatin.[8] Because proliferating cell lines do not precisely mimic the quiescent state of the cells that harbour latent HIV-1 *in vivo*, we developed a latency model in primary resting human CD4+ T cells transduced with the pro-survival gene *bcl-2* and used this model to screen for compounds that reverse latency.[9] Using this system, we screened more than 5000 compounds from the Johns Hopkins Drug Library and the MicroSource Spectrum Library (MicroSource Discovery Inc, CN, USA), and reported two latency-reversing agents, juglone (5-hydroxynaphthalene-1,4-dione)[9] and disulfiram.[10] Here we present a novel group of structurally related compounds that reactivate latent HIV-1 in the *bcl-2*-transduced primary CD4+ T cell model.

Materials and methods

Generation of bcl-2-transduced latently infected primary CD4+ T cells

This study was approved by the Johns Hopkins Institutional Review Board (NA_00049895). Healthy adult blood donors provided informed consent before enrolment. Latently infected cells were generated as described by Yang et al.[9] Briefly, primary CD4+ T cells were transduced with pro-survival gene *bcl-2*, which allows the cells to return to the resting state upon cytokine deprivation and ensures longer *in vitro* survival. Latency is established by infecting these *bcl-2*-transduced cells with a recombinant HIV-1 vector carrying a destabilized green fluorescent protein (GFP) gene in its *env* ORF, then allowing the infected cells to return to a resting memory state in a process that recapitulates the generation of resting memory CD4+ T cells *in vivo*. Following reversal of latency, newly expressed GFP can be detected by flow cytometry.

Measurement of latency reversal

Latently infected *bcl-2*-transduced resting CD4+ T cells were plated at 5×10^4 cells/well, or J-Lat cells were plated at 2.5×10^4 cells/well, in 200 μL of RPMI 1640 + 10% FBS in U-bottomed 96-well plates, and treated with stimuli for 24 h at 37°C. Cells treated with 10 μg/L phorbol 12-myristate 13-acetate (PMA) and cells treated with 2.5 mg/L anti-CD3 plus 1 mg/L anti-CD28 antibodies co-stimulation were used as positive controls. Reactivation of latent HIV-1 was determined by quantifying % GFP+ cells using a FACS Calibur flow cytometer (BD Biosciences, USA). Results were normalized to the response to co-stimulation or PMA treatment.

Measurement of toxicity

Freshly isolated primary CD4+ T cells were treated with quinolin-8-ol derivatives, or left untreated, for 24 h. Then cells were stained with propidium iodide (PI), and the percentage of viable (PI negative) cells was measured using flow cytometry. Gating the viable cells in forward versus side scatter plots produced very similar results.

Physical properties

Molecular weights, predicted octanol/water partition coefficients (miLogPs), and polar surface areas of hit compounds were calculated by online tools at www.molinspiration.com.

Measurement of cytokine release

Primary resting CD4+ T cells were treated with active quinolin-8-ol derivatives at their optimal concentration, or co-stimulation, or left untreated for 48 h. Concentrations of cytokines in cell culture supernatant were measured using a Meso Scale Human TH1/TH2 10-Plex Cytokine Array (Meso Scale Discovery, Gaithersburg, MA, USA).

Results and discussion

During the screening of the MicroSource Spectrum library, three compounds sharing the same quinoline core structure were identified as hits (Figure 1a). We designated quinolin-8-ol, which represents the common core structure, as hit A. The other two hits, 5-chloroquinolin-8-ol and 5-chloroquinolin-8-yl acetate, were designated as hits C1 and E0, respectively. A basic quinoline structure with position numbers is shown in Figure 1(b). Figure 1(c) shows representative flow cytometry data for these compounds from the screening. In forward scatter–side scatter (FCC-SCC) dot plots, cells treated with these hit compounds retained the same small size of the untreated resting CD4+ cells, while the positive control stimulus PMA induced T cell activation and a significant increase in cell size. The percentages of GFP+ cells (R2 in GFP-FL2 dot plots) induced by these hits were not as high as those with PMA treatment, but still highly significant ($P < 0.0001$) compared with the untreated cells.

We assembled a library of 46 derivatives of quinolin-8-ol, some from commercial sources but mostly by synthesis (S. Bhat, J. S. Shim, F. Zhang, C. R. Chong and J. O. Liu, manuscript submitted), and screened them using the primary cell model of latency. Based on the screen results, we synthesized and tested additional related compounds, and identified two

Figure 1. (a) Screening with a primary cell model of HIV-1 latency identified quinoline analogue hits. The results were normalized to the response to 10 μg/L PMA. (b) Basic quinoline structure. (c) A representative set of flow cytometry data for untreated cells, cells treated with compound A, and PMA-treated cells. The dot plots in the first row are FSC-SSC plots. The GFP-FL2 dot plots in the second row (red) show viable cells gated in R1 in FSC-SSC plots. Cells in R2 (GFP+) are reactivated latently infected cells. The % GFP+ cell is the number of cells in R2 divided by number of cells in R1. All flow data were collected under the same instrument settings. (d, e) Structures of two classes of analogues of the initial hits. (f, g) Dose–response curves for these two classes of quinoline derivatives in the primary cell model. Cells were treated with quarter-log serial dilutions of each compound for 24 h at 37°C. Results were normalized to the response to co-stimulation. Data are means of duplicate samples from one of three independent experiments using cells from different donors, all of which produced similar results.
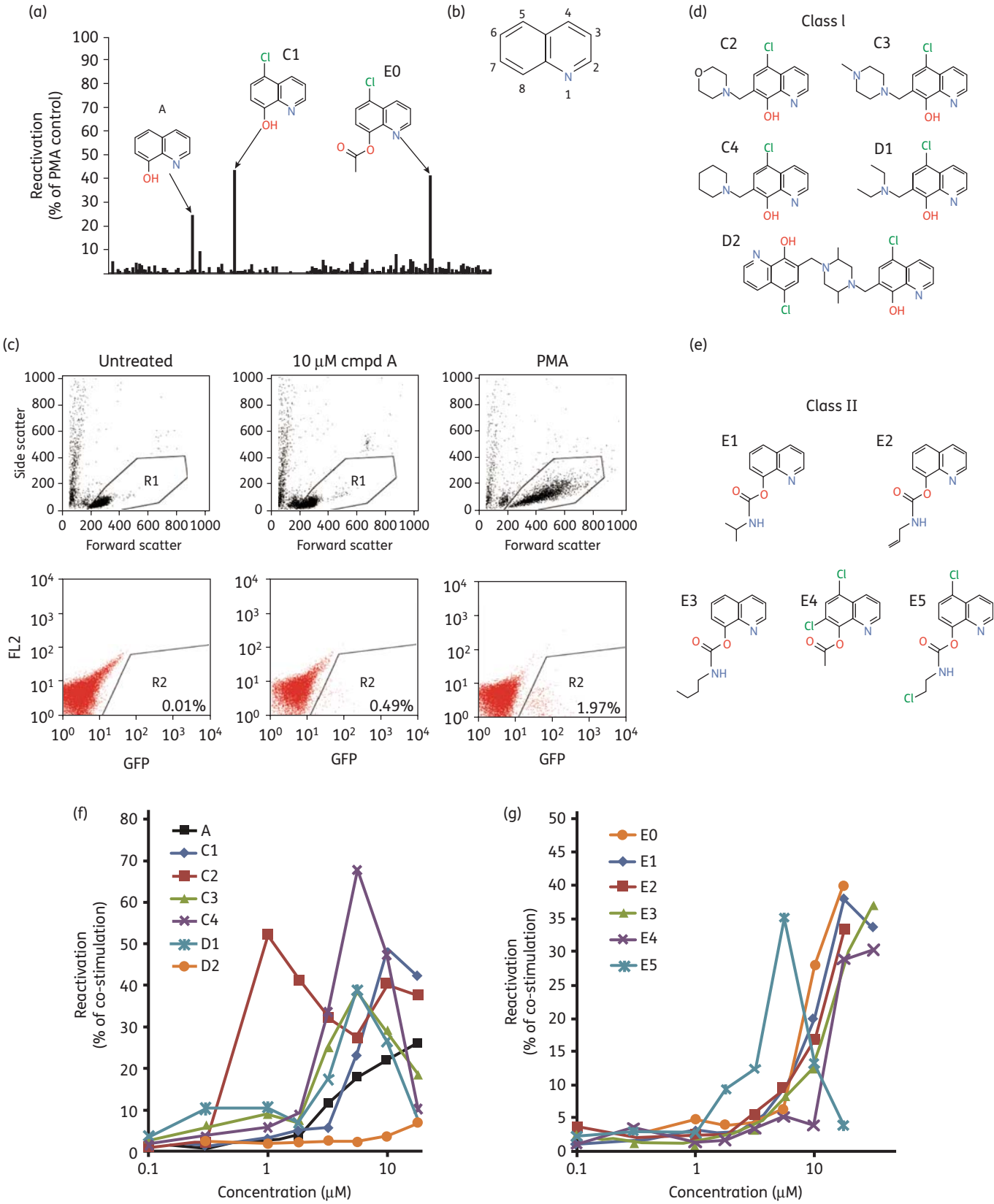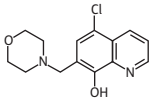
(a)

(b)

(c)

Untreated — 10 μM cmpd A — PMA

(d) Class I

(e) Class II

(f)

(g)
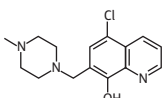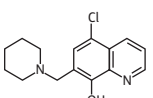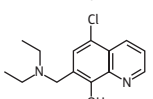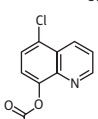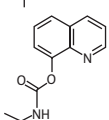
Table 1. Summary of activity and physical properties of the compounds
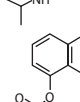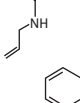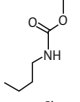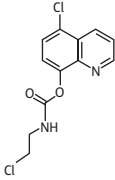
	Structure	Maximum activation (% of co-stimulation)	Concentration required for maximum activation (μM)	$EC_{50} \pm SD$ (μM)	Viable cells after maximal activation $\pm$ SD (%)	Viable cells at $EC_{50} \pm SD$ (%)	Molecular weight (Da)	Octanol/ water partition coefficient (miLogP)	Polar surface area ($\mathring{A}^2$)
A		26 ± 6	18	4.4 ± 1.2	93 ± 4	93 ± 4	145.161	1.675	33.12
C1		47 ± 5	10	5.6 ± 2.2	78 ± 8	91 ± 8	179.606	2.608	33.12
C2		55 ± 4	1	0.56 ± 0.1	87 ± 10	96 ± 3	278.739	2.186	45.592
C3		37 ± 3	5.6	2.5 ± 0.2	92 ± 7	97 ± 6	291.782	2.231	39.596
C4		68 ± 8	5.6	3.2 ± 0.3	76 ± 9	92 ± 6	276.767	3.248	36.358
D1		39 ± 4	5.6	3.3 ± 0.2	71 ± 8	88 ± 13	264.756	3.092	36.358
E0		40 ± 2	18	8.1 ± 0.3	84 ± 4	92 ± 4	221.643	2.379	39.197
E1		38 ± 4	18	10 ± 0.5	85 ± 13	91 ± 8	230.267	3.746	51.224
E2		33 ± 3	18	10 ± 0.3	88 ± 8	92 ± 7	228.251	1.918	51.224
E3		37 ± 8	32	13.5 ± 1.5	89 ± 8	94 ± 7	244.294	2.711	51.224
E4		30 ± 8	18	13.8 ± 0.8	81 ± 6	91 ± 3	256.088	2.985	39.197

Continued

Table 1. *Continued*

Structure	Maximum activation (% of co-stimulation)	Concentration required for maximum activation (μM)	$EC_{50} \pm SD$ (μM)	Viable cells after maximal activation ± SD (%)	Viable cells at $EC_{50} \pm$ SD (%)	Molecular weight (Da)	Octanol/ water partition coefficient (miLogP)	Polar surface area (Å^2)
E5	35 ± 3	5.6	3.9 ± 0.3	88 ± 9	96 ± 3	285.13	2.812	51.224

Summary of activity and physical properties of the compounds. Data are the mean ± SD of three independent experiments using cells derived from different donors. Cells were treated with quarter-log dilutions of the indicated stimuli for 24 h. Levels of activation were measured in latently infected *bcl-2*-tranduced primary resting CD4+ T cells as percentage of GFP$^+$ cells, normalized to co-stimulated cells. Toxicity was measured in freshly isolated primary CD4+ T cells as percentage of viable cells, normalized to untreated cells.

classes of quinolines that reactivate latent HIV-1 (Figure 1d and e). Class I compounds, designated C2–C4 and D1–D2, are the Mannich adducts of 5-chloroquinolin-8-ol. Class II compounds, designated E1–E5, are quinolin-8-yl carbamates, except E4, which is a simple acetate. We examined the dose–response relationships for these derivatives and the original hits A, C1 and E0 in the primary cell model (Figure 1f and g). Some derivatives, notably C2, induced activation at lower concentrations than the original hits. C2 and C4 appeared to be the most potent, followed by C3, D1, E5 and C1. D2 showed only minimal activity and was excluded from further studies.

The reactivation activity in the primary cell model, toxicity, and physical properties such as miLogP and polar surface area of the compounds are summarized in Table 1. Most EC_{50} values are in the 0.5–10 μM range, comparable to disulfiram in the same model. Some patterns are apparent in the structure–activity relationships. Among class I compounds (C2–C4), the presence of morpholine or piperidine rings at position 7 of the quinoline ring substantially lowers the concentration needed for activation, and in some cases increases the level of activation. D1, which is a Mannich adduct formed from an acyclic amine, is not as potent as C2 or C4. The bis-Mannich adduct D2 shows only minimal activity, as mentioned above. We also found that adding a phenyl group to the β-carbon of the Mannich adduct (for example compound C2 carrying such a phenyl group) eliminated the activity, suggesting some steric constraints in this region (data not shown). In addition, the presence of a chloro group at position 5 of the quinoline ring could possibly contribute to lowering the concentration needed for maximal activation. We also confirmed the ability of these compounds to reverse latency in another well-established model, J-Lat cells (Table S1, available as Supplementary data at *JAC* Online). Structure–activity relationships in J-Lat cells were very similar to those observed in the primary cell model, except that the concentrations needed for maximal activation and EC_{50} values were about one half-log lower.

All quinolin-8-ol derivatives that reactivate latent HIV-1 follow Lipinski's Rule of Five. Each contains no more than four hydrogen bond acceptors, and no more than a single hydrogen bond donor. Molecular weights for all of the active analogues tested are less than 300, and the calculated miLogPs are less than 4 (Table 1). These compounds also fulfil the criteria of Veber's Rules, as each has no more than five rotatable bonds and less than 60 Å^2 polar surface area (Table 1). Therefore these compounds are drug-like, and most of them qualify for the stricter 'rule of three' for leads, and thus have the potential for chemical modifications to improve activity and reduce toxicity while retaining drug-like characteristics.

Since agents that induce latent HIV-1 through global T cell activation would be too toxic for clinical use, mainly due to the cytokines secreted, we tested whether these compounds would induce cytokine secretion. None of the analogues induced detectable secretion of any of a panel of 10 cytokines (Table S2).

In conclusion, we discovered a group of quinolin-8-ol derivatives that can induce latent HIV-1 in a primary cell model without causing a substantial degree of T cell activation. This work expands the number of known latency-reversing agents and provides new scaffolds for drug development research. Although it is not yet clear whether these compounds can reactivate latent HIV-1 *in vivo*, it is possible that these compounds, or their derivatives, may be useful in future eradication strategies, either alone or in combination with other treatments.

Transparency declarations

All authors: none to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac/oxfordjournals.org/).

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