Quindoline-derivatives display potent G-quadruplex-mediated antiviral activity against herpes simplex virus 1

Ilaria Frasson a, Paola Soldà a, Matteo Nadai a, Martina Tassinari a, Matteo Scalabrin a, Vijay Gokhale c, Laurence H. Hurley b, Sara N. Richter * a

a Department of Molecular Medicine, University of Padua, Padua, Italy
b College of Pharmacy, University of Arizona, Tucson, AZ, 85721, United States
c BIOS Institute, University of Arizona, Tucson, AZ, 85721, United States

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A B S T R A C T

G-quadruplexes (G4s) are non-canonical nucleic acid structures that regulate key biological processes, from transcription to genome replication both in humans and viruses. The herpes simplex virus-1 (HSV-1) genome is prone to form G4s that, along with proteins, regulate its viral cycle. General G4 ligands have been shown to hamper the viral cycle, pointing to viral G4s as original antiviral targets. Because cellular G4s are also normally present in infected cells, the quest for improved anti-HSV-1 G4 ligands is still open. Here, we evaluated a series of new quindoline-derivatives which showed high binding to and stabilization of the viral G4s. They displayed nanomolar-range anti-HSV-1 activity paralleled by negligible cytotoxicity in human cells, thus proving remarkable selectivity. The best-in-class compound inhibited the viral life cycle at the early times post infection up to the step of viral genome replication. In infected human cells, it reduced expression of ICP4, the main viral transcription factor, by stabilizing the G4s embedded in ICP4 promoter. Quindoline-derivatives thus emerge as a new class of G4 ligands with potent dual anti-HSV-1 activity.

1. Introduction

Guanine quadruplexes (G4s) are non-canonical nucleic acid structures, which are being being widely investigated for their unique structural and functional features (Spiegel et al., 2020). G4s form in G-rich DNA strands: four guanine residues joined through Hoogsteen hydrogen bonds constitute a G-tetrad; G4s form when two or more G-tetrads stack on top of each other, coordinated by cations. Physiological cations, such as K+ and Na+, are the most relevant G-tetrads stabilizers. G4s display different conformations (i.e. parallel, antiparallel or mixed), which depend on the spatial orientation of adjacent guanine tracts, as well as on the length and nucleotide composition of connecting nucleotides, which are referred to as loops (Bryan and Baumann, 2011; Burge et al., 2006; Pipier et al., 2021).

G4s play pivotal roles at the human genome level (Hänsel-Hertsch et al., 2017), acting as switchers of crucial cellular processes such as transcription, replication, DNA repair and others (Varshney et al., 2020). More recently, G4s have been predicted and reported in the genome of microorganisms (Saranathan and Vivekanandan, 2019). In particular, G4s exert key functions in numerous DNA and RNA viruses infecting humans and at different stages of the viral life cycle (Lavezzo et al., 2018; Ruggiero et al., 2021; Ruggiero and Richter, 2018). Indeed, targeting viral G4s for antiviral purposes has been proposed and validated in vitro against several viruses (Ruggiero et al., 2021; Ruggiero and Richter, 2018).

The Herpes Simplex Virus 1 (HSV-1) infection is largely distributed in the worldwide adult population (~80%), being associated with both oral/genital sores as well as herpetic blindness and meningoencephalitis (“STD Facts - Genital Herpes,” 2020; World Health Organization, 2022). HSV-1 infection has also been involved in the onset of the Alzheimer disease (Cairns et al., 2022; Eimer et al., 2018; Itzhaki et al., 2020; Marcocci et al., 2020; Piacentini et al., 2014; Rizzo, 2020; Shinomoto et al., 2021). Treatments are based exclusively on nucleoside analog-based drugs, such as acyclovir and its derivatives (Seley-Radtke and Yates, 2018): they are efficient and display low toxicity, but resistance traits are rapidly emerging (Chen et al., 2021). Thus, development

Abbreviations: CC50, Half Maximal Cytotoxicity; G4, G-quadruplex; hpi, hours post infection; HSV-1, Herpes Simplex Virus type 1; IC50, Half Maximal Inhibitory Concentration; ICP4, Infected Cell Polypeptide 4; MOI, Multiplicity Of Infection.

* Corresponding author.

E-mail address: sara.richter@unipd.it (S.N. Richter).

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of novel drug-like molecules, with new mechanisms of action against HSV-1 infection are urgently needed. Multiple G4 clusters are present in the HSV-1 genome, both in repeat regions and in promoter of immediate early genes that control the viral life cycle; HSV-1 G4s also mediate the association of viral proteins to the viral genome (Artusi et al., 2015, 2016; Frasson et al., 2019, 2021). G4-ligands displayed anti-HSV-1 activity based on inhibition of viral replication (Artusi et al., 2015; Gallego et al., 2017).

To search for improved anti-HSV-1 activity, we here tested a novel class of Quinoline-derived compounds. Quinoline is a derivative of the natural product cryptolepine, which binds and stabilizes G4s in the c-myc promoter (Boddupally et al., 2012; Dai et al., 2011; Dickerhoff et al., 2021; Ou et al., 2011). We evaluated their ability to selectively inhibit the HSV-1 viral cycle. Compound GSA-0932 showed remarkable antiviral properties and negligible toxicity in human host cells. It drastically reduced the binding to G4s (Table 2). These compounds were synthesized from the common intermediate 11-chloroindoloquinoline 1 (Brown et al., 2011). GSA-0820 is a quinoline derivative and was synthesized from the parent quindoline and impart novel structural features that would in

2. Materials and methods

The complete description of this section is available in Supplementary Information.

3. Results

3.1. Chemistry

GQC-05, an ellipticine derivative was identified as G4 binding agent (Brown et al., 2011). GSA-0820 is a quinoline derivative and was synthesized from the common intermediate 11-chloroindoloquinoline 1 (Table 1) (Boddupally et al., 2012; Miranti et al., 2020). GSA-0932 and related analogs were developed with an aim to disrupt planarity of the parent quinoline and impart novel structural features that would increase the binding to G4s (Table 2). These compounds were synthesized starting from 11-chloroindoloquinoline using two step synthesis (Scheme 1). One of the major features in the synthesis is the cyclization step to create seven membered cyclic system in the quinoline structure. Different analogs carrying a variety of side chains with varying properties were synthesized and tested.

3.2. Quinoline-derived compounds display significant anti-HSV-1 activity

We initially tested quinoline-derivatives, and quinoline as parent compound, for their antiproliferative activity against the human U-2 OS cell line, which is permissive to HSV-1 infection (Deschamps and Kalamvoki, 2017; Frasson et al., 2021). The compounds’ cytotoxic effect was calculated as the concentration that reduced U-2 OS cells viability by 50%, with respect to the vehicle-treated cells, and was expressed as cytotoxic concentration (CC50) (Table 3, Fig. S1). Antiviral activity was assessed and reported as inhibitory concentration (IC50), i.e. the compound concentration able to reduce the viral titre by 50% with respect to the vehicle-treated infected cells (Table 3). The antiviral assays were performed at compounds’ concentrations that displayed less than 20% cytotoxic activity on host cells. For each compound, both CC50 values and cell viability curves in the presence of increasing concentrations of compounds were reported (Table 3 and Fig. 1A–K). The selectivity index (SI) of each compound was calculated as the ratio of CC50 over IC50 values (Table 3): the higher the SI value, the more effective and less toxic the antiviral drug (U.S. Food and Drug Administration, 2006). The viral titre reduction obtained at the highest tested concentration (400 nM) was also reported (Table 3).

Most compounds displayed considerable antiviral activity, with IC50 values in the nanomolar range. The parent compound quinoline, GSA-0932 and GSA-1502 displayed the highest anti HSV-1 activity, with similar inhibitory concentrations (~160 nM) (Table 3 and Fig. 1A, G, I). GQC-05, GSA-0932 and GSA-1202 induced viral inhibition higher than 70% at the highest tested concentration (Table 3 and Fig. 1B, G, H). GSA-0820, GSA-0825, GSA-0903, and GSA-0920 displayed low cytotoxicity values in the nanomolar range. The parent compound quindoline, GSA-0820, GSA-0825, GSA-0903, and GSA-0920 displayed low cytotoxicity to the four compounds (Table 3 and Fig. 1C-F). Some compounds, as GSA-1202 and GSA-1504, did not display a strict dose-dependent response curve, possibly due to aggregation issues, and thus were not

Table 1

| Compound | Structure | Chemical Name |
|----------|-----------|---------------|
| GSA-0820 | 2-(4-(10H-indolo[3,2-b]quinolin-11-yl)piperazin-1-yl)-N,N-diethyl-1,2-diamine | N1,N2-diethyl-N1,N2-(10H-indolo[3,2-b]quinolin-11-yl)ethane-1,2-diamine |
| GQC-05   | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |

Table 2

| Compound | R1 | Chemical Name |
|----------|----|---------------|
| GSA-0825 | 4,3-(2,3-dihydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluoren-1(1H)-yl)propyl | N1,N2-diethyl-N1,N2-(10H-indolo[3,2-b]quinolin-11-yl)ethane-1,2-diamine |
| GSA-0903 | 4-(3-(4-methylpiperazin-1-yl)propyl)-1,2,3,4-tetrahydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |
| GSA-0920 | 4,3-(azepan-1-yl)propyl)-1,2,3,4-tetrahydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |
| GSA-0932 | 1-(2-(2,3-dihydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene-4,1H)-yl)ethyl | piperedin-4-ol |
| GSA-1202 | 4,3-(piperidin-1-yl)propyl)-1,2,3,4-tetrahydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |
| GSA-1502 | 2-(2,3-dihydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene-4,1H)-yl)-N,N-dimethylpropan-1-amine | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |
| GSA-1504 | 3-(2,3-dihydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene-4,1H)-yl)-N,N-dimethylthian-1-amine | 2-((5,11-dimethyl-6H-pyrido[4,3-b]carbazol-9-yloxy)-N,N-dimethylthian-1-amine |
| GSA-1512 | 4-(2-(2,3-dihydro-4,9,13b-triazabenzo[b]cyclohepta[4H]fluorene-4,1H)-yl)ethyl | cyclohexan-1-ol |
considered further. GSA-0932 displayed the highest SI, coupled to the highest reduction of viral yield at the highest tested concentration (77%, Table 3) and was thus selected for further investigation.

### 3.3. Quindoline derivative GSA-0932 selectively stabilizes HSV-1 G4s

To characterize GSA-0932 binding and selectivity towards HSV-1 G4s, circular dichroism thermal stability analysis was performed. Both conformational and stability changes were taken into consideration. Three representative and previously characterized HSV-1 G4s were used: un2 is embedded in the terminal repeat of the HSV-1 genome, gp054a resides in the coding region of the gp054 gene, which encodes for the essential tegument protein UL36, un3 is embedded in the promoter of the Infected Cell Polypeptide 34.5 (ICP34.5) gene (Artusi et al., 2015; Dai et al., 2011). In our assay, the reference drug was acyclovir (ACV), a thoroughly characterized inhibitor of HSV-1 DNA polymerase (Elion, 1983; Gnann et al., 1983; Taylor and Gerriets, 2021). U-2 OS cells were infected (MOI 1) and treated with GSA-0932 (12.375 μM) of GSA-0932 induced Taq polymerase stalling, implying that GSA-0932 antiviral effect was induced by a block of DNA polymerase activity.

#### Table 3

Anti-HSV-1 activity and cytotoxicity of the Quindoline-derived compounds. Summary table reporting the tested compound antiviral properties, indicated as Inhibitory Concentration 50 (IC50, compound concentration required to inhibit 50% of HSV-1 viral titre), in relation to the Cytotoxic Concentration 50 (CC50, compound concentration able to reduce host cell viability by 50%). The ratio between the IC50 and the CC50 is the Selectivity Index (SI).

| Compound      | U-2 OS cell line | IC50 (nM)   | CC50 (nM)   | SI   | Viral titre reduction (%) at the highest tested concentration |
|---------------|------------------|------------|------------|------|-------------------------------------------------------------|
| Quindoline    |                  | 155.9 ± 6.7| 2200.2 ± 489.3| 14.1 | 68                                                          |
| GQC-05        |                  | 267.4 ± 21.8| 1654.6 ± 118.5| 6.2  | 72                                                          |
| GSA-0820      | >400             | 2984.8 ± 7.5| 171.2      | 45   |                                                             |
| GSA-0825      | 356.9 ± 60.9     | 32709.2 ± 313.8| 91.6  | 56   |                                                             |
| GSA-0903      | >400             | 5205.8 ± 13.0| 492.8     | 12.5 |                                                             |
| GSA-0920      | >400             | 3620.6 ± 9.1| 92.1   | 45   |                                                             |
| GSA-0932      | 165.0 ± 49.1     | 19953.5 ± 738.0| 117.5 | 77   |                                                             |
| GSA-1202      | 248.0 ± 64.4     | 4852.9 ± 261.3| 19.6  | 73   |                                                             |
| GSA-1502      | 155.49 ± 27.6    | 14758.5 ± 1067.4| 95.2  | 60   |                                                             |
| GSA-1504      | 308.7 ± 82.2     | 4509.6 ± 516.8| 14.6  | 65   |                                                             |
| GSA-1512      | 255.6 ± 114.3    | 18089.5 ± 389.8| 70.8  | 69   |                                                             |

#### Scheme 1

Synthesis of quindoline analogs.
μM), corresponding to 75-fold their calculated/reported IC₅₀ values for strain F HSV-1 (Crute et al., 2002; Daelemans et al., 2011), the highest possible concentration devoid of cytotoxicity (Chu et al., 2020; Honda et al., 2001). Compounds were added from T₀ (time of infection) up to T₁₀ (10 h post infection, hpi), by 2 h intervals.

ACV-treatment of HSV-1-infected U-2 OS cells showed pronounced viral titre reduction when the compound was administrated up to 8 hpi, whereas increasing viral titres were detected afterwards. This is in line with the reported ACV mechanism of action, as the drug targets viral DNA polymerase during viral DNA replication that occurs between 6 and 12 hpi (Ibañez et al., 2018; Weller and Coen, 2012). GSA-0932 hindered viral cycle progression when administrated up to 8 hpi. Up to 6 hpi, GSA-0932 blocked HSV-1 infection more potently than ACV. These data suggest that GSA-0932 inhibits viral replication similarly to ACV (Everett, 2014), and also that GSA-0932 could act at earlier stages. Indeed, key regulatory G4s in the promoters of the immediate early viral genes have recently been reported (Frasson et al., 2019, 2021).

HSV-1 IE genes are necessary to prime the host cells for viral gene expression: among them, the major HSV-1 transcription factor, ICP4, regulates viral transcription via G4 binding and unfolding, and it concurrently regulates its own expression recognizing the G4s embedded in its own promoter (Frasson et al., 2021). We thus tested GSA-0932 for its ability to downregulate ICP4 expression via G4 stabilization. To test this hypothesis, we first checked GSA-0932 binding to the four G4s embedded in the ICP4 promoter (Figs. S5–S6 and Table S2).

U-2 OS infected cells (MOI 1) were treated with GSA-0932 or ACV at increasing concentrations (0–10 μM). ICP4 expression levels (mRNA copies) were evaluated at 6 hpi (Fig. 4B), hence at the time of the highest ICP4 expression and prior to massive viral replication, (Dremel and DeLuca, 2019). ICP4 transcription levels were normalized by the human TATA-binding protein (TBP) expression level (Radonić et al., 2005). Both GSA-0932 and ACV reduced ICP4 expression at all the tested concentrations but, compared to ACV, GSA-0932 was remarkably more potent, as it almost completely abolished ICP4 transcription at the highest tested concentrations (2.5, 5 and 10 μM). ICP4 protein levels were assessed by Western Blot analysis in the same conditions (Fig. 4C). GSA-0932 induced significant dose-dependent reduction of ICP4 expression that exceeded 95% at the highest compound concentration (Fig. 4D). These data indicate that GSA-0932 inhibits the HSV-1 viral cycle by also acting at early stages, i.e. hampering the expression of ICP4, the major viral IE protein.

To further characterize GSA-0932 antiviral activity, its distribution in the nucleus of HSV-1- and mock-infected cells was examined. First, GSA-0932 UV-visible and fluorescence spectra were recorded (Fig. S7). The compound displayed absorption peaks at 419, 346, 332, and 286 nm, and a main emission peak at 458 nm (Fig. S4), which allowed its detection in human cells by diode laser. GSA-0932 widely distributed in the nucleus of mock-infected cells upon 2 h treatment at augmenting concentrations (4–8 μM) (Fig. S8A–C). Punctate aggregations of GSA-0932 were visible in some cells, which may correspond to nucleoli.
and nucleolin accumulation (Miranti et al., 2020). No difference in compound cellular distribution was detected at the tested concentrations.

To study GSA-0932 cellular distribution during HSV-1 infection, a

![Fig. 2](image_url) Thermal unfolding on the HSV-1 G4 sequences folded in 2.5 mM KCl in the absence and presence of GSA-0932. (A) un3 G4 alone and (B) with GSA-0932; (C) un2 G4 alone and (D) with GSA-0932; (E) gp054a G4 alone and (F) with GSA-0932. Spectra were recorded over a temperature range of 20–90 °C. Oligonucleotide folding was tested in two independent assays, one replicate per condition. Representative spectra per each oligonucleotide are shown. Arrows indicate the direction of changes in molar ellipticity.

Table 4  
Melting temperatures (\(T_m\)) of HSV-1 G4 sequences in the absence/presence of GSA-0932. \(T_m\) values (°C) were calculated according to the van ‘t Hoff equation. KCl concentrations are indicated. SD indicates standard deviation, nd stands for “not determined”.

| Oligonucleotide | 2.5 mM K⁺ | 100 mM K⁺ |
|-----------------|-----------|-----------|
| \(T_m\) (°C)     | \(\Delta T_m \pm SD\) (°C) | \(T_m\) (°C) | \(\Delta T_m \pm SD\) (°C) |
| un3             | 37.6 ± 0.4 | 65.0      | 56.4 ± 1.2 | 66.4 ± 1.4 |
| un3 + GSA-0932  | 56.4 ± 0.4 | 18.8 ± 0.8 | 66.4 ± 1.4 | 1.4 |
| un2             | 82.4 ± 1.2 | >90       |           |           |
| un2 + GSA-0932  | >90       | >7.5      | >90       | nd        |
| gp054a          | 57.3/82.4 | 58.9/    |           |           |
| gp054a + GSA-0932 | >90 | >7.5 | >90 | 15.6 |

Table 5  
Relative binding affinity analyzed by MS competition assay for un3, un2, gp054a, hTel, and c-myc G4 oligonucleotides. nd stands for not determined (due to the technical impossibility to correctly assign MS peaks to the respective samples).

| Competing G4s | un3 | un2 | gp054a | Cell G4 (hTel or c-myc) |
|---------------|-----|-----|--------|------------------------|
| un3/hTel      | 50.3 ± 1.2 | 13.8 ± 0.17 |
| un3/c-myc     | 37.1 ± 0.77 | 59.5 ± 1.1 |
| un2/hTel      | 38.9 ± 1.70 | 11.1 ± 0.36 |
| un2/c-myc     | 29.6 ± 1.9 | 46.2 ± 1.59 |
| gp054a/hTel   | 57.3 ± nd | 45.6 ± 0.43 |
| gp054a/c-myc  | 51.8 ± nd | 45.6 ± 0.75 |
DNA replication sites were observed (Fig. 5B) (Artusi et al., 2016, 2021; La Boissière et al., 2004). In infected cells, GSA-0932 signal distributed in the nucleus (Fig. 5A) with distinctive clusters, which co-localized with HSV-1 VP16 protein, thus indicating that the compound accumulates in the typical nuclear areas where the new viral genomes are produced (Fig. 5A–C) (Artusi et al., 2016, 2021; La Boissière et al., 2004).

These data indicate that GSA-0932 efficiently hampers HSV-1 replication by hindering the viral cycle up to the replication steps, with an initial G4-mediated downregulation of the major IE genes, followed by accumulation in the nuclear viral replication compartments with subsequent impediment of viral DNA replication.

4. Discussion

In the present work, we tested quindoline-derived G4 ligands as a novel class of G4 ligands for their ability to selectively target HSV-1 G4s (Artusi et al., 2015; Frasson et al., 2019, 2021; Lavezzo et al., 2018). Conventional G4-ligands are often characterized by large aromatic scaffolds with reported administration and selectivity issues (Balasubramanian et al., 2011; Ruggiero and Richter, 2018). The drug-likeness of the quindoline-derived G4 ligands and their reduced cytotoxicity in human cells make them attractive molecules for antiviral purposes (Boddupalle et al., 2012; Ou et al., 2019). Indeed, most of the tested quindoline-derivatives displayed significant antiviral activity against HSV-1, in the nanomolar range of concentrations. Most candidates exhibiting promising selectivity indexes, due to negligible cytotoxicity in human host cells. Some compounds (i.e. GSA-1202 and GSA-1504) did not display a strict dose-dependent response curve, possibly due to aggregation issues, and thus were not further considered. GSA-0932 offered the best combination of potent anti-HSV-1 activity and high selectivity (SI). Both activity and selectivity data on GSA-0932 analogs show interesting structure-activity relationship (SAR) trends. i) Compounds with two carbon linkers between seven-membered ring and R1 group (GSA-0932, GSA-1502, GSA-1512) display higher SI values. ii) Compounds with three carbon linkers (GSA-0903, GSA-0920, GSA-1202 and GSA-1504) show lower SI values. iii) GSA-0932 consists of both positively charged basic nitrogen and hydrogen bonding group (-OH) for its interaction with phosphate backbone of quadruplex structure.

GSA-0932 displayed excellent affinity for the viral G4s and it hampered HSV-1 viral cycle acting up to the viral replication step. Since we have previously observed that IE gene expression is modulated by multiple G4s embedded in IE gene promoters (Artusi et al., 2015, 2016; Frasson et al., 2019, 2021), we investigated and observed remarkable affinity of GSA-0932 on the expression of ICP4, HSV-1 main transcription factor, at the early stages of viral infection. In contrast, ACV displayed only minor effect on ICP4, thus confirming that ACV acts on viral genome replication (Liu et al., 2021; Shen et al., 2019). Hence, GSA-0932 by acting at G4s both reduced HSV-1 genome replication, similarly to other G4 ligands (Artusi et al., 2015, 2021; Callegaro et al., 2017) and acyclovir (Elion, 1983), and restricted ICP4 gene/protein expression.

GSA-0932 was able to bind and stabilize different G4s: it showed remarkable affinity towards HSV-1 structures but maintained good binding also to c-myc G4 (King et al., 2020; Miranti et al., 2020), while displaying low affinity for telomeric G4s. In infected cells, viral G4s rapidly overcome the amount of cell G4s, fact that may explain the highly selective antiviral activity of GSA-0932 (Artusi et al., 2015, 2016; Frasson et al., 2019, 2021). However, binding of GSA-0932 to c-myc G4 might also improve its antiviral activity: it has been reported that HSV-1 modulates c-myc expression in infected cells (Alfonso-Dunn et al., 2017; Birkenheuer et al., 2018; Hilton et al., 1995; Hu et al., 2016; MacLeod and Minson, 2010; Polpitiya Arachchige et al., 2018), so that likely augment cell permissiveness to the virus. GSA-0932 binding to c-myc G4 drops oncogene transcription in human cells (Hilton et al., 1995), so that GSA-0932 may affect HSV-1 replication in infected cells both by hindering viral transcription and replication through direct interaction at viral G4s and by disrupting the cellular response prompted by the virus.

![Fig. 3. Taq polymerase stop assay on HSV-1 G4s. (A) Un2, gp054a, un3 templates were amplified by Taq polymerase in the absence (lanes 1,5,13) and presence of K+, combined with increasing amounts (2–8 μM) of GSA-0932 (lanes 7–8, 11–12, 15–16) or the same amount of DMSO as that in the ligand (lanes 6,10,14). Un3 was analyzed at 50 mM of K+, while the other sequences were investigated at 0.5 mM of K+. A template (non-G4 ctrl) unable to fold into G4 was also used as control (lanes 1–4). P stands for unreacted labeled primer, FL stands for full-length product. G4-specific Taq polymerase stop sites are highlighted by vertical bars. (B) Full-length and G4 stop bands intensity quantification relative to the Taq polymerase stop assay shown in panel (A).](image-url)
to allow its replication.

5. Conclusions

The described data on quindoline-derivatives confirmed the crucial role played by G4s in the HSV-1 viral cycle and pointed out how G4 ligands can be used as effective and innovative tools in the management of viral infections. The possibility that G4 ligands recognize both the viral target G4s and cellular G4s which help increase the antiviral activity, will allow the development of innovative antiviral strategies that will lead to novel cutting-edge therapeutic approaches in the treatment of crucial human viral-related diseases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.
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