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Structural optimization of a retrograde trafficking inhibitor that protects cells from infections by human polyoma- and papillomaviruses

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ABSTRACT

Human polyoma- and papillomaviruses are non-enveloped DNA viruses that cause severe pathologies and mortalities. Under circumstances of immunosuppression, JC polyomavirus causes a fatal demyelinating disease called progressive multifocal leukoencephalopathy (PML) and the BK polyomavirus is the etiological agent of polyomavirus-induced nephropathy and hemorrhagic cystitis. Human papillomavirus type 16, another non-enveloped DNA virus, is associated with the development of cancers in tissues like the uterine cervix and oropharynx. Currently, there are no approved drugs or vaccines to treat or prevent polyomavirus infections. We recently discovered that the small molecule Retro-2^{cycl}, an inhibitor of host retrograde trafficking, blocked infection by several human and monkey polyomaviruses. Here, we report diversity-oriented syntheses of Retro-2^{cycl} and evaluation of the resulting analogs using an assay of human cell infections by JC polyomavirus. We defined structure–activity relationships and also discovered analogs with significantly improved potency as suppressors of human polyoma- and papillomavirus infection in vitro. Our findings represent an advance in the development of drug candidates that can broadly protect humans from non-enveloped DNA viruses and toxins that exploit retrograde trafficking as a means for cell entry.

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1. Introduction

Polyomaviruses are small, non-enveloped, viruses with an icosahedral capsid that contains a double-stranded DNA genome.[1] These viruses have established latent infections in the vast majority of the human population.[1,2] Primary infection often occurs early in life, and it is estimated that as much as 90% of the adult population is seropositive for BK polyomavirus (BKPyV) and as much as 40% of adults are seropositive for JC polyomavirus (JCPyV).[2] Polyomaviruses establish an asymptomatic infection in humans, with polyomavirus-associated disease seen only in the context of immunosuppression, such as in AIDS patients or during immunomodulatory therapy. During circumstances of reduced immune function, increased replication and dissemination of JCPyV can lead to the development of the neurodegenerative disease progressive multifocal leukoencephalopathy (PML), which affects 3–5% of AIDS patients.[3,4] BKPyV-associated disease is most often observed during immunomodulatory therapy, and can lead to the development of hemorrhagic cystitis and polyomavirus-associated nephropathy in as many as 10% of transplant patients.[1,5] Human papillomaviruses (HPVs) are also non-enveloped, DNA viruses.[6] HPV infection and replication is limited to the squamous epithelial tissue[7] and is believed to be associated with as much as 5% of all cancers, most notable of which are cancer of the cervix, other anogenital tissue, and the oropharynx.[6,8] Prophylactic vaccination against certain types of HPV has been successful. However, HPV related diseases will remain a significant human health problem for at least several decades for individuals who refuse vaccination or who become infected before being vaccinated. Currently, there are no approved small-molecule therapeutics for the treatment or prevention of PyV and HPV infection. Therapeutic strategies that help to manage the spread of these viruses would have significant value in medicine.

The development of antiviral agents is often guided by consideration of viral life cycles. Many non-enveloped viruses, including PyV and HPV, are unable to access the host cytoplasm directly from

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the cell surface or from endosomes after endocytosis.[7,9] They therefore tend to exploit host vesicular trafficking en route to the Golgi apparatus or the endoplasmic reticulum, from which they are released into the cytoplasm before reaching the nucleus for replication.[7,9–13] The movement of virus particles, macromolecules, and metabolites from the cell surface to the endoplasmic reticulum via the Golgi is known as retrograde trafficking. This phenomenon is an important intracellular transport mechanism wherein protein, lipids, and small molecules are transported from endosomes to the trans-Golgi network and Golgi membranes.[11] Retrograde trafficking is the primary mechanism for recycling chaperones, receptors, and other cargo molecules that are targeted to the cell membrane from the Golgi. In principle, small molecule modulation of host intracellular trafficking could serve as a useful strategy for the prevention of infections by non-enveloped viruses. Indeed, we recently reported that Retro-2^{cycl}, a dihydroquinazolinone (DHQZ) inhibitor of retrograde trafficking[14,15], blocked the infection of cells by human and monkey polyomaviruses[16] as well as by human papillomaviruses.[17]

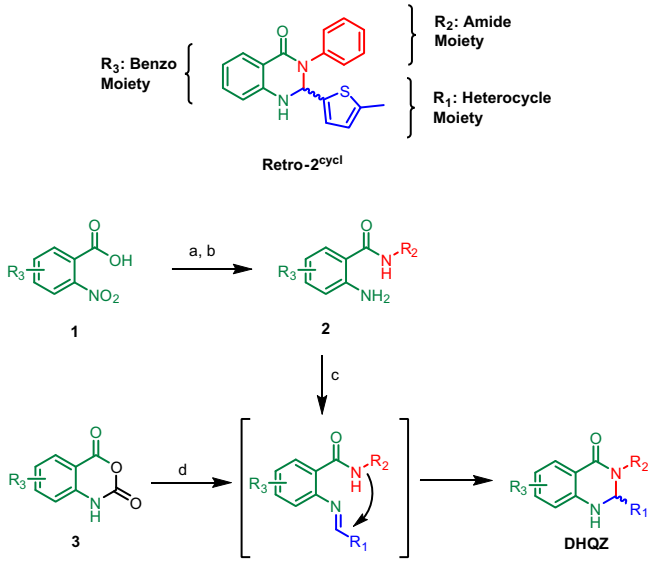
Retro-2^{cycl} apparently blocks toxin and viral retrograde transport without significantly affecting endogenous trafficking.[14–17] However, the specific host cellular factor that is targeted by Retro-2^{cycl} is not yet known. Here, we have used a diversity-oriented synthetic strategy to prepare DHQZs that are structurally related to Retro-2^{cycl}. The capacities of the compounds to inhibit the infection of human cells by JCPyV were assessed systematically. The experiments revealed critical structure activity-relationships (SAR) and led to the discovery of Retro-2^{cycl} analogs with enhanced capacities to prevent both JCPyV and HPV infections.

2. Results

2.1. Structure–activity relationship (SAR) analysis of the dihydroquinazolinone Retro-2^{cycl}

The suppression of virus infection by Retro-2^{cycl} encouraged us to pursue a SAR analysis to define critical structural elements for bioactivity. We anticipated that such an analysis would also yield compounds with superior potency as retrograde trafficking inhibitors. For this analysis, we divided the Retro-2^{cycl} structure into three distinct elements (Scheme 1) a heterocycle moiety, an amide moiety, and a benzo moiety. We therefore carried out compound diversification in consecutive phases wherein one moiety was varied independently of the other two. After each phase, the most active compound served as a new lead structure for the subsequent diversification phase. The activities of the compounds prepared in each stage were systematically assessed in assays wherein human SVGA cells were infected with JCPyV.

Two routes were used for the diversity-oriented synthesis of DHQZs (Scheme 1). In one route, primary amines were coupled to 2-nitrobenzoic acid (**1**) using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine; subsequently, the nitro group was reduced by transfer hydrogenation with ammonium formate and 10% palladium on carbon in methanol to afford anthranilamides (**2**). The anthranilamide intermediates were condensed with aromatic aldehydes in the presence of scandium(III) triflate under microwave irradiation to afford the desired DHQZs. Alternatively, DHQZs were prepared in a one-pot, tandem reaction sequence comprised of the decarboxylative condensation of an isatoic anhydride (**3**) and primary amines in THF, followed by the scandium(III) triflate-catalyzed condensation of the resulting amides with aromatic aldehydes. Preparation of DHQZs from isatoic anhydrides (**3**) was the preferred route. However, in certain instances, the availability of starting materials or the weak nucleophilicity of primary amines (like anilines) necessitated preparation of DHQZs from 2-nitrobenzoic acids (**1**).



Scheme 1. Reagents and conditions: (a) R_2NH_2, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, dichloromethane, room temperature, 16 h; (b) 10% Pd/C, ammonium formate, methanol; (c) R_1CHO, $Sc(OTf)_3$, methanol, MW 100 °C, 1 h; (d) R_2NH_2, tetrahydrofuran, reflux, then R_1CHO, $Sc(OTf)_3$.

The first moiety to be analyzed was the heterocycle substituent of the dihydroquinazolinone aminal carbon (Table 1). In particular, three structural aspects of the heterocycle moiety were investigated: the identity of the heteroatom, the ring substitution pattern, and the identity of the ring substituent. Compounds **4**–**6**, which are substituted with an unsubstituted thiophene, a pyrrole, or a furan, all exhibited significantly attenuated anti-JCPyV activity relative to Retro-2^{cycl}. Interestingly, compound **7**, bearing a 5-methylfuran moiety, had similar activity to that of Retro-2^{cycl}, which itself bears a 5-methylthiophene moiety. Although the 5-methylthiophene moiety confers greater potency, it is apparent that a 5-methylfuran moiety can also be tolerated. The pattern and identity of the thiophene substituent also proved to be significant with respect to bioactivity. Compound **8**, bearing a methyl group at the thiophene 4 position, had nearly equal activity to that of Retro-2^{cycl}. On the other hand, the activity of compound **9**, bearing a methyl group at the 3 position of the thiophene, was attenuated to the same extent as Retro-2^{cycl} analogs with unsubstituted heterocycle moieties. An analog that had a 5-ethyl thiophene (compound **10**) in place of the parent compound's 5-methyl thiophene had markedly improved potency. Interestingly, activity is severely compromised when the heterocycle is a benzothiophene ring (compound **11**). Therefore, the 5-ethylthiophene moiety was held constant throughout the remainder of the SAR analysis.

We next turned our attention to the amide moiety (Table 2) and the effects of various aliphatic and aromatic amide groups on JCPyV infectivity. Most of the compounds with substituents on the amide nitrogen had activities that were similar those of compound **10** and compound **12**, which has only a hydrogen substituent. Dramatic improvements in activity were observed in compounds wherein the phenyl group of Retro2^{cycl} was replaced by either benzyl (compound **17**) or methylnapthyl (compound **19**) groups. In the context of SAR analysis, our finding that the incorporation of a benzyl group onto the amide moiety improved potency was fortuitous because many substituted benzylamines are commercially available. Using readily available building blocks, we were able to prepare many DHQZs with structurally diverse

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