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Dihydropyrimidinones and -thiones with improved activity against human polyomavirus family members

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ABSTRACT

Human polyomaviruses are generally latent but can be reactivated in patients whose immune systems are suppressed. Unfortunately, current therapeutics for diseases associated with polyomaviruses are non-specific, have undefined mechanisms of action, or exacerbate the disease. We previously reported on a class of dihydropyrimidinones that specifically target a polyomavirus-encoded protein, T antigen, and/or inhibit a cellular chaperone, Hsp70, that is required for virus replication. To improve the antiviral activity of the existing class of compounds, we performed Biginelli and modified multi-component reactions to obtain new 3,4-dihydropyrimidin-2(1*H*)-ones and -thiones for biological evaluation. We also compared how substituents at the N-1 versus N-3 position in the pyrimidine affect activity. We discovered that AMT580-043, a N-3 alkylated dihydropyrimidin-2(1*H*)-thione, inhibits the replication of a disease-causing polyomavirus in cell culture more potently than an existing drug, cidofovir.

Polyomaviruses are double stranded DNA viruses that are endemic in the human population but are usually not disease-causing. However, in individuals who are immune-compromised, select members of this virus family can be reactivated and cause serious ailments.[1,2] For example, BK virus reactivation in renal transplant patients undergoing immunosuppressive therapies leads to BK virus associated nephropathy (BKVAN), which is observed in 5–10% of all kidney transplant recipients. More than half of these individuals will ultimately lose the donated organ. BK virus reactivation is also evident in cancer patients who undergo bone marrow transplants and are given immunosuppressants, leading to hemorrhagic cystitis. Similarly, 5–10% of HIV-infected individuals ultimately succumb to progressive multifocal leukoencephalopathy (PML), which arises from JC virus reactivation. Due to recent improvements in virus identification and sequencing techniques, the number of known polyomaviruses has increased, such that there are now 12 human polyomavirus types. Several of these new polyomavirus family members have also been linked to disease.[3] Unfortunately, existing therapeutics to treat polyomavirus infections are non-specific and/or exhibit unwanted side-effects.[4] Cidofovir (CDV), an FDA approved treatment for cytomegalovirus (CMV) retinitis in AIDS patients, is an acyclic dCMP analog that inhibits DNA polymerase and is commonly used off-label to treat polyomavirus infections (Fig. 1).[4] Brincidofovir (BCV, CMX001) is a prodrug of CDV and shares its mechanism of action.[5] A cell based high-throughput screen for simian virus 40 (SV40) and polyomavirus (BK and JC) inhibitors also detected activity with ellagic acid and spiperone.[6]

We previously reported on the synthesis, screening, and preliminary structure–activity relationship (SAR) studies of multi-component reaction-derived dihydropyrimidinones that inhibit the growth of polyomaviruses, in particular MAL2-11B, BQU015242, LR340-006, ML282-56, and ML282-86 (SMAL) (Fig. 1); we also identified hexachlorophene and bithionol as polyomavirus inhibitors.[7–11] The most specific dihydropyrimidinone compounds were identified based on their ability to inhibit the ATPase activity of T antigen, which is a polyomavirus-encoded

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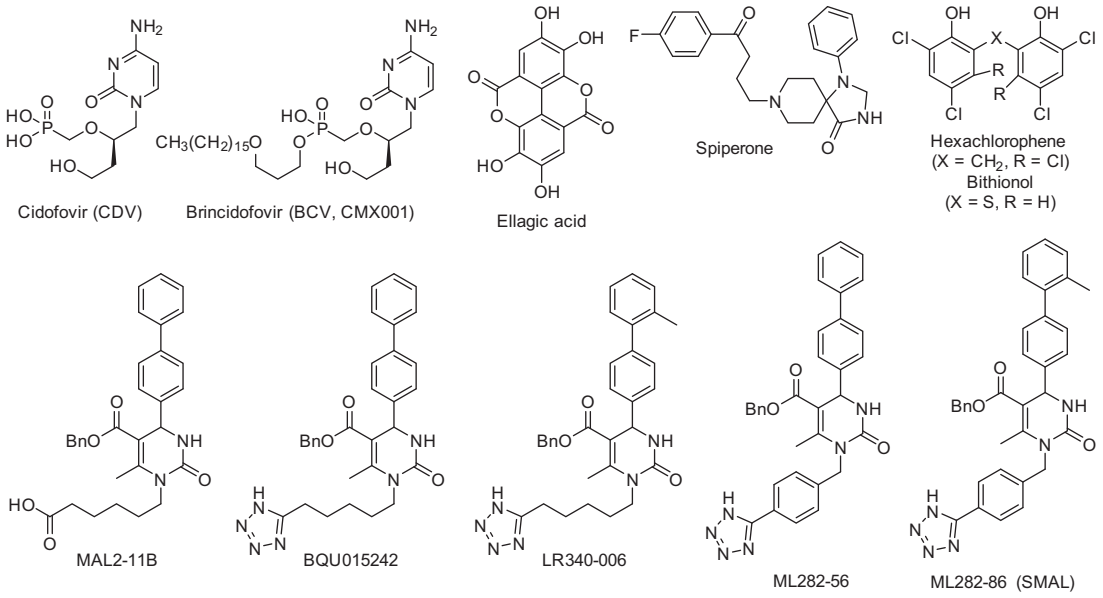

Figure 1. Inhibitors of BK polyomavirus replication and propagation.

factor. T antigen lacks human homologs and is required to catalyze replication of the polyomavirus genome.[12] Some of our polyomavirus inhibitors also compromised the ability of T antigen, which contains a region with homology to Hsp40 chaperones, to activate the ATPase activity of the Hsp70 molecular chaperone. Hsp70 is required for T antigen-catalyzed polyomavirus replication.[13,14] Our most potent compound, SMAL,[7] inhibited T antigen ATPase activity with an IC_{50} of 5 μM. However, SMAL was as effective in cell-based viral replication assays as compounds that were less potent in the in vitro ATPase assay. Therefore, in order to identify improved inhibitors of polyomavirus replication, we now synthesized and characterized a class of SMAL variants that bear a thiourea group in place of the urea moiety in the dihydropyrimidinones (Fig. 2). This modification allowed us to investigate the influence of electronic and physicochemical inhibitor properties on antiviral activity, such as: (1) the greater electron-density and polarizability of the thiocarbonyl compared to the carbonyl group; (2) the modification of H-bond donor (HBD) and H-bond acceptor (HBA) properties in the thiourea versus urea, and (3) the increased acidity of the thiourea NH group versus the amide NH.[15,16] In addition, we switched key moieties at the N-1 and N-3 positions in the heterocycle to determine whether altered placement of active side chains affects T antigen and Hsp70 inhibition.

The synthesis of N-1 substituted 3,4-dihydropyrimidine-2(1*H*)-thiones was achieved through a series of Biginelli reactions (Scheme 1).[17–19] Thioureas **2a–c** were obtained in 61–78% yield by condensation of amines **1** with thiocarbonyldiimidazole (TCDI) in the presence of triethylamine, followed by aminolysis with aqueous ammonia. According to a procedure described by Tolmachev and co-workers,[20] **2c** was sonicated in the presence of [1,1′-biphenyl]-4-carbaldehyde, benzyl 3-oxobutanoate and TMSCl for 1 h and subsequently stirred at rt for several days, followed by saponification with 1 N NaOH to yield the desired Biginelli acid **3e** (AMT551-090). In an analogous fashion, thioureas **2a** and **2b** were converted to the heterocycle-linked nitriles which were further modified with trimethylsilylazide and TBAF to give the corresponding tetrazoles **3a–d**.

The synthesis of the regioisomeric N-3 alkylated analogs of dihydropyrimidinethiones **3** used the Atwal modification of the Biginelli reaction (Scheme 2). Enones **4** were condensed with 2-(4-methoxybenzyl)isothiouronium chloride (**5**) in the presence of sodium acetate in DMF to give the desired isothioureas **6** in 64–81% yield. Regioselective N-3 alkylation[21] with bromonitrile and bromoester electrophiles gave the dihydropyrimidines **7a–c** in 54–62% yield. Nitriles **7a** and **7b** were converted to tetrazoles **8a** and **8b**, respectively, whereby tetrazole formation with TMS-azide

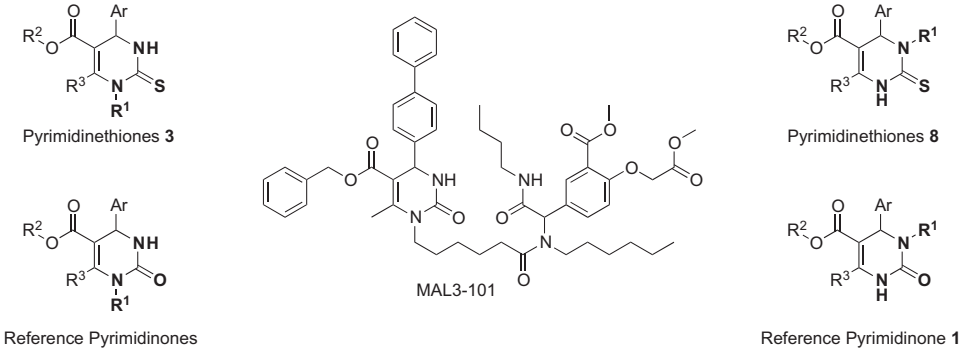

Figure 2. Structures of heterocyclic cores probed in this work and compound MAL3-101, a reference pyrimidinone along with **12** (AMT628-003), MAL2-11B, BQU015242, LR340-006, ML282-56, and ML282-86 (SMAL). See Supplemental materials for an overview table that lists, in alphabetical order, all compound codes and the corresponding structures for all assayed analogs, as well as their sequential compound numbers from the synthetic schemes and their corresponding UPCMLD codes.

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