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(54) Title: ANTIVIRAL COMPOUNDS AND METHODS FOR TREATING INFECTIONS CAUSED BY DOUBLE-STRANDED DNA VIRUSES

(57) Abstract: The present invention relates to polyamide compounds and their use in pharmaceutical compositions and in medical applications for the treatment of human papillomavirus infections and/or polyomavirus infections.

ANTIVIRAL COMPOUNDS AND METHODS FOR TREATING INFECTIONS CAUSED BY DOUBLE-STRANDED DNA VIRUSES

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND OF THE INVENTION

[0002] The present invention relates to polyamide compositions and therapies for treatment of cells and tissues infected with double stranded DNA viruses, for example, human papillomaviruses (HPV) and/or polyomaviruses (PyV).

[0003] A significant part of the viral life cycle of many DNA viruses has them maintained as double-stranded, closed circular, supercoiled, chromatinized DNAs within the nucleus. Among these viruses are a number of types that cause a significant disease burden including papillomaviruses, polyomaviruses, Epstein Barr virus (EBV), and hepatitis B virus (HBV), a member of the genus *Orthohepadnavirus.*

[0004] Polyomaviruses (PyV) are a family of small (~5 KB), non-enveloped DNA viruses that occupy a broad replicative niche in vertebrates from birds to humans, but individually have a narrow species tropism. Simian virus 40 (SV40), an archetypal PyV was first discovered as a tumor-inducing virus in rhesus monkey kidney cells cultures(1) and subsequently became an important tool for studying DNA replication, repair, and oncogenesis. Recent years have seen considerable growth of the number of known polyomaviruses, with human polyomaviruses (HPyV) now numbering over ten(2). HPyV

are suspected etiological agents in a number of cancers, but only MCPyV has been definitively associated with the rare and aggressive Merkel Cell carcinoma(3).

[0005] PyV often persist as latent infections without causing disease, but reactivation of the infection may lead to disease in a variety of tissues. For example, reactivation of PyV in immunocompromised patients is a growing area of concern. BK polyomavirus (BKV) is the major cause of polyomavirus-associated nephropathy (PyVAN) causing 1-15% of kidney transplant patients to be at risk of premature allograft failure(4). There are a growing number of PyVAN patients due to increases in the numbers of renal transplants and the development of more effective immunosuppressive drugs(5). Likewise, 10-25% of bone marrow transplant (BMT) patients are susceptible to hemorrhagic cystitis of the bladder and lower urinary tract that is largely attributable to BKV reactivation due to immunosuppression(6).

[0006] Progressive multifocal leukoencephalopathy (PML) is a rare, but typically fatal, inflammation of the white matter of the brain in multiple locations that is attributable to JC polyomavirus (JCV)(7). PML is typically associated with JCV reactivation in severely immunocompromised patients such as those receiving immunosuppressive therapy following transplant, following chemotherapy, or in those with AIDS. Other HPyV are also associated with morbidity following reactivated in immunocompromised patients. Merkel cell polyomavirus (MCPyV), for example, has been implicated as the etiological agent in Merkel cell carcinoma, a cancer with a strikingly high incidence in AIDS; chronic lymphocytic leukemia (CLL) patients; and in immunosuppressed organ transplant patients, including but not limited to bone marrow transplant recipients(7). There are currently few antiviral treatment options for polyomaviruses and so antiviral therapies would be an important advancement for diseases associated with these viruses(7).

[0007] HBV is a small (~3.2 KB), enveloped DNA virus that infects hepatocytes and replicates by way of an RNA intermediate(8). The encapsidated viral genome consists of 3.2 kB of relaxed, circular DNA which is converted to covalently, closed, circular DNA

(cccDNA) upon its translocation to the nucleus. Chronic infection by HBV contributes to a number of diseases of the liver including hepatitis with progression to cirrhosis and hepatocellular carcinoma(9). More than 240 million people are infected with HBV, and 780,000 people die annually due to the consequences of HBV infection(10). There is no specific treatment for HBV although some patients can be treated with Interferon or antiviral agents to slow the progression of the disease. Seven drugs have been licensed by the FDA, to date, for the treatment of chronic hepatitis B infection: interferon-alpha and pegylated interferon-alpha, three nucleoside analogs (lamivudine, entecavir and telbivudine) and two nucleotide analog prodrugs (adefovir dipivoxil and tenofovir disoproxil fumarate)(11). Current antiviral agents can control but not eliminate HBV because HBV establishes a stable nuclear cccDNA. These drugs have little impact upon the levels of cccDNA and, therefore, while helpful to control infection, have little effect upon long-term HBV persistence. Strategies to eliminate cccDNA may prove very helpful for treatment of hepatitis due to chronic HBV infection(12-14).

[0008] Human papillomavirus (HPV) is a small double-stranded DNA virus that colonizes various stratified epithelia like skin, oral and genital mucosa, and induces the formation of self-limiting benign tumors known as papillomas (warts) or condylomas. Most of these benign tumors naturally regress due to the influence of host immunological defenses. Some HPVs, however, have oncogenic potential and have been associated with certain types of cancers. See, Lorincz et al., Obstetrics & Gynecology, 79:328-337 (1992); Beaudenon et al., Nature, 321:246-249 (1986); and Holloway et al., Gynecol. One., 41:123-128 (1991).

[0009] HPV is the most prevalent, sexually transmitted virus. More than 35 HPV genotypes are known to be sexually transmitted, but a subset accounts for the majority of ano-genital infections. Among these most common HPV types are two forms with high risk for carcinogenic progression (HPV16 and HPV18), and two forms that cause the majority of genital warts (HPV6 and HPV11).

[0010] An estimated 5.5 million people become infected with HPV each year in the

United States, and an estimated 20 million Americans are currently infected (Cates and et al., Lancet, 354, Suppl. SIV62, 1999). Approximately 75 percent of the male and female reproductive-age population has been infected with sexually transmitted HPV and, though the main public health risk to women is cervical cancer (Koutsky, Am. J. Med., 102(5A), 3-8, 1997), genital warts constitute an epidemic. Thus, millions of people in the U.S. alone require treatment each year. It is important to note that PAP smears represent the largest public health screening program in the world, and that the test is, essentially, a measure of HPV infection. One standard for managing a positive PAP smear is "follow up". In general, no treatment is recommended unless an advanced stage of cervical dysplasia is observed (CDC Sexually Transmitted Diseases Treatment Guidelines, 2002).

[0011] Significant need exists in HPV positive subjects for effective HPV antiviral drugs. At present, no specific treatments exist for HPV or warts. Aldara.TM. (Imiquimod), an immunomodulator used for treating external genital warts, is the most successful treatment on the market. An effective, specific HPV treatment has the potential to significantly improve upon, and effectively compete with, Imiquimod.

[0012] The majority of human cervical carcinomas (95%) contain and express HPV DNA and it is the expression of two viral oncoproteins, E6 and E7 that appears to be critical for cellular transformation and maintenance of the transformed state. Specifically, four HPV types (HPV-16, HPV-18, HPV-31, and HPV-45) have been connected to 75-93% of the cases of cervical cancer in the United States. It has been estimated that perhaps twenty percent (20%) of all cancer deaths in women worldwide are from cancers that are associated with HPV.

[0013] HPV also causes anal cancer, with about 85 percent of all cases caused by HPV-16. HPV types 16 and 18 have also been found to cause close to half of vaginal, vulvar, and penile cancers.

[0014] Most recently, HPV infections have been found to cause cancer of the

oropharynx, which is the middle part of the throat including the soft palate, the base of the tongue, and the tonsils. In the United States, more than half of the cancers diagnosed in the oropharynx are linked to HPV-16.

[00015] HPVs can be further classified as either high or low risk based on the clinical lesions with which they are associated or the relative propensity for these lesions to progress to cancer. Low risk cutaneous types, such as HPV types HPV-1, HPV-2, HPV-3, HPV-4, HPV-5, HPV-7, HPV-8, and HPV-9 cause common warts (verrucae vulgaris), plantar warts (verrucae plantaris), mosaic warts, flat warts (verrucae plane), and butcher warts. Furthermore, HPV types HPV-6 and HPV-11 cause warts of the external genitalia, anus and cervix. High-risk types, such as HPV-16, HPV-18, HPV-31, HPV-33 and HPV45 are particularly common in intraepithelial carcinomas, neoplasias and cancers. In particular, the genomes of two HPV types, HPV-16 and HPV-18, have been found to be associated with about 70 invasive carcinomas of the uterine cervix, as well as cancers of the oro-pharynx, anus, and other mucosal tissues.

[00016] Current treatment for HPV infection is extremely limited. Management normally involves physical destruction of the wart by surgical, cryosurgical, chemical, or laser removal of infected tissue. Some of these current treatments, like laser removal and surgery, are expensive and require the use of anesthesia to numb the area to be treated. Cryosurgical removal requires the use of special equipment. Furthermore, most subjects experience moderate pain during and after the procedure.

[00017] Topical creams and solutions such as preparations of 5-fluorouracil, Imiquimod, cidofovir, formaldehyde, glutaral, cimetidine, tricholoroacetic acid, bleomycin, podofilox and podophyllum preparations have also been used. (Reichman in Harrison's 7 Principles of Internal Medicine, 13th Ed. (Isselbacher et al., eds.); McGraw-Hill, Inc., NY (1993) pp. 801-803). Recurrence after these treatments, however, is common, most likely because the virus remains latent within the host epithelial cells. Therefore, subsequent repetitive treatments must be used, which can destroy healthy tissue. These treatments are not available or approved for treatment of cervical infections.

[00018] Interferon has also been employed as a treatment for persistent HPV infections and warts. However, its effectiveness is limited. Chang et al. (2002) Journal of Virology 76: 8864-74, found some cells infected with HPV genomes became resistant to interferon treatment after only a few applications. See also Cowsert (1994) Intervirol. 37:226-230; Bornstein et al. (1993) Obstetrics Gynecol. Sur. 4504:252-260; Browder et al. (1992) Ann. Pharmacother. 26:42-45.

[00019] Thus, there is a need for therapeutics for treating a number of diseases and conditions as outlined herein.

BRIEF SUMMARY OF THE INVENTION

[00020] The present invention provides compounds and methods for treating subjects who have been infected with a double-stranded DNA virus, for example, human papillomavirus or polyomavirus. The polyamide compounds of the invention are useful as treatments for papillomavirus or polyomavirus related diseases. In some embodiments, the polyamide antiviral agents are well suited for treating PyVAN, hemorrhagic cystitis and PML. In some embodiments, the polyamide antiviral agents are well suited for treating cervical epithelia and anal epithelia, conjunctiva papillomas, condyloma accumulata and recurrent respiratory papillomatosis (RRP).

[00021] Chemical compositions known as polyamides, and their formulations for cell culture, tissue culture and use against high-risk human papillomavirus (HPV16, 18 and 31), are described in U.S. Patent Application Publication No. 2009/0306164, U.S. Patent Application Publication No. 2013/0090362, as well as U.S. Patent No. 7,589,171, U.S. Patent No. 8,119,677 and U.S. Patent No. 8,993,609. These polyamide compounds, compositions and formulations are herein incorporated by reference to the aforementioned disclosures in their entirety.

[00022] The present invention provides polyamides, polyamide compositions, and analogs or derivatives thereof, and methods for treating polyomavirus infected cells and

methods for treating papillomavirus infected cells by administering the polyamides of the invention.

[00023] The present invention provides a method of treating polyomavirus or papillomavirus infections, including infected subjects, cells, organs tissues, and the like, referred to hereafter as infected entities, comprising contacting the infected entities according to a manner described here, or using a related treatment common to pharmaceutical and biological practice.

[00024] In an embodiment, the invention provides a method of treating human papillomavirus or polyomavirus infected entities comprising administering to a patient or subject a polyamide compound or pharmaceutical composition comprising a polyamide compound described herein. In an aspect of the invention, the method further comprises contacting the infected entities with an anti-viral agent in addition to the polyamide compound.

[00025] In an embodiment, the polyomavirus may be selected from SV40, BKV, JCV, KI polyomavirus, MCPyV, WU polyomavirus or polyomavirus 9.

[00026] In another aspect, the HPV may be selected from HPV1, HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 or HPV68.

[00027] The present invention further provides a method of treating virus infected cells comprising contacting the cells with an effective amount of a polyamide in accordance with the invention. The virus may be selected from HPV, polyomaviruses, or other double-stranded DNA viruses. A subject infected with HPV may be treated by a method, which comprises administering to the subject an effective amount of a polyamide having a structure as described herein. The polyamide compound may be administered in the form of a pharmaceutical composition comprising the compound and a pharmaceutically acceptable carrier. In other embodiments, the invention provides a method of treating

double-stranded DNA virus infected cells comprising administering to a patient a compound selected from the classes described herein.

[00028] Examples of polyamide compounds are generically and specifically described herein.

[00029] In an embodiment, the invention provides a method of treating a subject exhibiting or who is suspected of exhibiting HPV infected cells or polyomavirus infected cells by administering to the subject a compound selected from compounds listed in Table 1.

[00030] In an embodiment, the method further comprises administering antiviral agents selected from cidofovir, CMX-001, leflunomide, adefovir, entecavir, lamivudine, telbivudine, tenofovir, interferon, pegylated interferon, and/or drugs effective against hepatitis B, in combination with the polyamides of the invention for the treatment of subjects/patients exhibiting or suspected of exhibiting a HPV, polyomavirus infection and/or other infections caused by double-stranded DNA viruses.

[00031] The methods of this invention exhibit efficacy against papillomavirus or polyomavirus related disease, or both, that is superior to other available antiviral treatments. This superior efficacy is due to the ability of the polyamide compounds to trigger elimination of DNA viral episomes. The polyamides are known to bind viral DNA directly, and this may be part of the trigger. The diseases benefitting from treatment include prevention of HPV-derived cancers and direct HPV infections, as well as polyomavirus related diseases including PML, PyVAN, hemorrhagic cystitis, and Merkel cell carcinoma.

[00032] Moreover, the HPV related diseases benefitting from treatment include genital or cutaneous warts, HPV infections of oral or genital tissues including cervical epithelia and anal epithelia caused by the HPV, conjunctiva papillomas, condyloma accumulata and recurrent respiratory papillomatosis (RRP).

[00033] In some embodiments, polyamide sequences exhibiting anti-HPV activity with the HPV types, especially, HPV 1, 6, 11, 16, 18 and 31, display the ability to displace or eliminate HPV DNA from host chromosomes, which can result in broad applicability against HPVs. These include HPV11, which is responsible, in part, for the frequently fatal disease known as respiratory papillomatosis, as well as genital warts, HPV1 and 6, which cause common warts and warts of the external genitalia, anus and cervix, respectively, and HPV16, 18 and 31, which are responsible for anal and/or cervical cancers.

[00034] Notwithstanding the foregoing, it may nonetheless not be predicted which double-stranded DNA (dsDNA) viruses are suitable targets for treatment with a therapeutically effective amount of the pharmaceutical compositions comprising the polyamides of the invention.

[00035] What we therefore believe to be comprised by our invention may be summarized *inter alia* in the following words.

[00036] A compound of the formula:

Guan-PPPβPPβPIm–γ–PβPPβPPPβPβTa;

TMG-PPβPPβPIm–γ_{NH2}–PβPPβPPPβPPTa;

Guan-PPPβPPβPIm–γ–PβPPβPPPβPβDp;

TMG-PβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβPIm–γ_{NHR}–PβPPβPPPβPβTa;

ImPPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβPIm–γ–PβPPβPPPβPβDp;

TMG-PPPβPPβPIm–γ–PβPPβPPPβPβTa;

Guan-PPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβP–γ–PPPβPPPβPβ-Ta;

ImPPβPPImβPP–γ–PPβPPPβPPPPTa;

TMG-PPPβPPβPIm–γ_{NH2}–PβPPβPPPβPβTa;

TMG-PPβPPβP–γ–PPPβPPPβPβTa;

Guan-IPPβPPIβPP–γ–PPβPPPβPPPβTa;

TMG-IPPβPPIβPP–γ–PPβPPPβPPPβTa;

ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβDp;

TMG-PPβPPImβPP–γ–PPβPPPβPPPβDp;

TMG-PPPβPPbP–$\gamma_{NHR'}$–PPPβPPPβPβDp;

ImPPβPPImβP–γ–PPβPPPβPPPβTa;

ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβTa;

TMG-PβPPImβPP–γ–PPβPPPβPPPβDp;

Guan-PPβPPImβPP–γ–PPβPPPβPPPβDp;

Guan-IPPβPPP–γ–PPβPPPPβTa;

ImPPβPPP–γ–PPβPPPPβTa;

TMG-IPPβPPP–γ–PPβPPPPβTa;

ImPPβPP–γ–PPβPPPPβTa;

ImPPβPPP–γ_{NH2}–PPβPPPPβTa;

ImPPβPPImβPP–γ_{NHAc}–PPβPPPβPPPβDp;

Guan-PPβPPP–γ–PPβPPPPβTa;

ImPPPβPPβ–γ–PPβPPPβPPDp;

TMG-PPβPPP–γ–PPβPPPPβTa; or

Guan-PPβPPP–γ–PPβPPPPβDp,

wherein GUAN = a guanidinyl radical; TMG = tetramethylguanidinyl;
P = 4-amino-2-carbonyl-N-methylpyrrole; γ = gamma-aminobutyric acid;

γ_{NH2} = (R)-2,4-diaminobutyric acid reacted through either the 2-amino group or the 4-amino group; γ_{NHAc} = (R)-2-(acetylamino)-4-aminobutyric acid;
β = beta-alanine; Im = 4-amino-2-carbonyl-N-methylimidazole;
Ta = 3,3'-diamino-N-methyldipropylamine; and Dp = (dimethylamino)propylamine, such a

[00037] compound which is in the form of a formate salt, such a

[00038] use of a compound in a medicament for the treatment of polyomavirus infected cells, such a

[00039] method of treating cells infected with a polyomavirus comprising administering to a subject infected with the polyomavirus, a therapeutically effective amount of a compound, such a

[00040] compound of the formula:

ImPPPβPP-γ-PPPβPPPβDp;
ImPPPβPPβPPP-γ_{NH2}-PPPβPPPβPPPβDp;
ImPPPβPPβPPP-γ-PPPβPPPβPPPβDp;
ImPPPβPPP-γ_{NH2}-PPPβPPPββDp;
ImPPPβPPβ-γ-PPβPPPβPβTa;
ImPPβPPP-γ-PPβPPPPβTa;
ImPPPβPPβ-γ_{NH2}-PPβPPPβPβDp;
ImPPβPPP-γ_{NH2}-PPβPPPPβTa;
ImPPPβPPP-γ-PPPβPPPββTa;
ImPPPβPPβ-γ_{NH2}-PPβPPPβPβTa;
ImPPβPImPβPP-γ_{NH2}-PPβPPPβPPPβTa;
ImPPβPPImβPP-γ-PPβPPPβPPPβDp;
ImPPβPPImβPP-γ-PPβPPPβPPPβTa;
ImPPPIm-γ-βPPPPβDp;
ImPPPIm-γ-βPPPPβTa;

ImPPβPImPβPPIm-γ-βPβPPPβPPPβTa;

ImPβPPβPPImβPPP-γ-PβPPβPPPβPPPPβTa;

ImPPPβPPPβPP-γ-PPPβPPPβPPPβDp;

ImPPPβPPPβPP-γ-PPPβPPPβPPPβTa;

ImPPβPPImβPPαPPβPPPβPPPβTa (5 TFA);

ImPPβPPImβPPαNH2PPβPPPβPPPβDp (4 TFA);

ImPPβPPImβPPαNHAcPPβPPPβPPPβTa (4 TFA);

ImPPPImαNH2βPPPPβTa (5 TFA);

ImPPβPPPαNHAcPPβPPPPβTa (3 TFA);

ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβDp;

ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβTa;

ImPPβPPImβPP-γ_{NHAc}-PPβPPPβPPPβDp;

ImPPβPPP-γ-PPβPPPPPTa • 3TFA;

ImPPβPPImβPP-γ-PPβPPPβPPPPTa • 4TFA;

TMG -PPβPPβPIm-γ_{NH2}-PβPPβPPPβPPTa • 5TFA;

TMG -PPPβPPβPIm-γ_{NHR}-PβPPβPPPβPβTa • 6TFA;

TMG -PPPβPPβPIm-γ_{NH2}-PβPPβPPPβPβTa • 5TFA;

TMG -PPPβPPβP-γ_{NHR}-PPPβPPPβPβTa • 5TFA;

TMG -PPPβPPβPIm-γ_{NH2}-PβPPβPPPβPβDp • 4TFA;

TMG -PPPβPPβP-γ_{NH2}-PPPβPPPβPβTa;

TMG -PPPβPPβP-γ_{NH2}-PPPβPPPβPβDp;

TMG –PβPPImβPP-γ-PPβPPPβPPPβTa • 4TFA;

TMG -PPPβPPβP-$\gamma_{NHR'}$-PPPβPPPβPβDp • 3TFA;

TMG –PβPPImβPP-γ-PPβPPPβPPPβDp • 3TFA;

TMG –PPβPPP-γ-PPβPPPPβDp • 2TFA;

TMG –PPβPPP-γ-PPβPPPPβTa • 3TFA;

TMG –PPβPPImβPP-γ-PPβPPPβPPPβTa;

TMG –PPβPPImβPP-γ-PPβPPPβPPPβDp;

TMG –PPPβPPβPIm-γ-PβPPβPPPβPβTa;

TMG –PPPβPPβPIm-γ-PβPPβPPPβPβDp;

TMG –PpβPPβPIm-γ-PβPPβPPPβPβDp;

TMG –PpβPPβPIm-γ-PβPPβPPPβPβTa;

ImPPβPP-γ-PPβPPPPβTa;

ImPPβPPImβP-γ-PPβPPPβPPPβTa;

TMG –PPβPPβP-γ_{NH2}-PPPβPPPβPβTa;

TMG –PpβPPβPIm-γ_{NH2}-PβPPβPPPβPβTa;

TMG –PPβPPβP-γ-PPPβPPPβPβ-Ta • 3TFA;

TMG –PPPβPPβP-γ-PPPβPPPβPβ-Ta • 3TFA;

Guan-PPβPPImβPP-γ-PPβPPPβPPPβTa (4 TFA);

Guan-PPβPPP-γ-PPβPPPPβTa (3 TFA);

Guan-PPβPPImβPP-γ-PPβPPPβPPPβDp (3 TFA);

Guan-PPβPPP-γ-PPβPPPPβDp (2 TFA);

Guan-PPPβPPβPIm-γ-PβPPβPPPβPβTa (4 TFA);

Guan-PPPβPPβPIm-γ-PβPPβPPPβPβDp (3 TFA);

TMG –IPPβPPP-γ-PPβPPPPβTa (4 TFA);

TMG –IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA);

Guan-IPPβPPP-γ-PPβPPPPβTa (4 TFA);

Guan-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA);

Ac-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA);

PPβPPβPIm-γ-PβPPβPPPβPβTa;

ImPPβPPP-γ-PPβPPPPβTa (3 HCO2H);

ImPPPβPP-γ-PPPβPPPβTa (3 HCO2H);

TMG –ImPβPPP-γ-PβPPPPβTa (4 HCO2H);

Guan-ImPβPPP-γ-PβPPPPβTa (4 HCO2H);

ImPβPPP-γ-PβPPPPβTa (3 HCO2H);

ImPPβPP-γ-PPβPPPβTa (3 HCO2H);

TMG –ImPPβPP-γ-PPβPPPβTa (4 HCO2H);

Guan-ImPPβPP-γ-PPβPPPβTa (4 HCO2H);

TMG –PPImβPP-γ-PPβPPPβTa (4 HCO2H);

Guan-PPImβPP-γ-PPβPPPβTa (4 HCO2H);

PPImβPP-γ-PPβPPPβTa (3 HCO2H);

ImPPβPPImβPP-γ-PPβPPPβPPPβTa (4 HCO2H);

ImPPPβPP-γ-PPPβPPPβDp (2 TFA);
ImPPβPPImβPP-γ-PPβPPPβPPPβTa-AF488; or
ImImPIm-γ-PβPPβTa-AF488 (2 HCO_2H),

wherein GUAN = a guanidinyl radical;TMG = tetramethylguanidinyl; P = 4-amino-2-carbonyl-N-methylpyrrole; γ = gamma-aminobutyric acid; γ_{NH2} = (R)-2,4-diaminobutyric acid reacted through either the 2-amino group or the 4-amino group; γ_{NHAc} = (R)-2-(acetylamino)-4-aminobutyric acid; β = beta-alanine; Im = 4-amino-2-carbonyl-N-methylimidazole;Ta = 3,3'-diamino-N-methyldipropylamine; Dp = (dimethylamino)propylamine; TFA = trifluoroacetic acid; HCO_2H = formate; α means that the γ-aminobutyric acid formed an α-linked hairpin rather than the typical γ-linked hairpin; and AF488= AlexaFluor-488 fluorophore, such a

[00041] compound which is in the form of a formate salt, such a

[00042] use of a compound in a medicament for the treatment of papillomavirus infected cells, such a

[00043] method of treating cells infected with a papillomavirus comprising administering to a subject infected with the papillomavirus, a therapeutically effective amount of the compound.

BRIEF DESCRIPTION OF THE FIGURES

[00044] Figure 1: : Light micrographs of Giemsa stained, adherent BSC-1 cells following infection with SV40 and following SV40 treatment and treatment with 10μM NV1042 at day 1 post-infection. Note that NV1042 cells are protected from SV40 infection.

[00045] Figure 2: Viral copies of SV40 per ng of DNA in adherent and floating cells in SV40 cells infected with SV40 (MOI = 1) or in infected cells treated with and single, 48

hour dose of 10µM NV1042 delivered on day 2 post-infection. Copies of SV40 / ng DNA are plotted on an exponential scale.

[00046] Figure 3: Time course as in Figure 1 showing expression of large T antigen (LT or LgT-ag, red) and appearance of activated phospho-ATM (pATM, green) in days post SV40 infection in BSC-1 cells that have received no compounds or been treated at day 1 post infection with NV1042. DAPI stained nuclei are in blue. Cells were infected with a multiplicity of infection (MOI) = 1.

[00047] Figure 4: Quantification of levels of total cells, pATM positive cells, and LTag cells from time course illustrated in Figure 3. Cells were infected with a multiplicity of infection (MOI) = 1.

[00048] Figure 5: The size of the DNA virus episome appears to correlate with polyamide activity. HPV16, SV40, and BKV-Tu and BKV-Dun strains all exhibit similar IC_{50}'s in response to NV1042 in the low nm range. The results indicate that NV1042 is practical for treating the small DNA viruses HPV, SV40, and BKV.

[00049] Figure 6: Pharmacologic inhibition of MRE11 sensitizes SV40 and BKV episomes to NV1042. The IC_{50} for NV1042 against SV40 and BKV is 32nM and 9nM respectively without Mirin (solid boxes), and 12nM and 5nM in the presence of 100µM Mirin (open diamonds). Treatment with 100µM Mirin alone had no effect on episome levels. n = 3 independent experiments, error bars represent the standard error of the mean. We reported the same effect for HPV: inhibition of MRE11 sensitizes HPV episomes to elimination by MV1042 (PA25). (Edwards TG, Vidmar TJ, Koeller K, Bashkin JK, Fisher C (2013) DNA Damage Repair Genes Controlling Human Papillomavirus (HPV) Episome Levels under Conditions of Stability and Extreme Instability. PLoS ONE 8(10): e75406. doi:10.1371/journal.pone.0075406).

[00050] Figure 7: Activity of polyamide and cell survival is promoted by inhibition of the Chk2 kinase which acts downstream of the ATM kinase during a DDR. As in the case

of Mirin (Fig. 6), the sensitivity of viral DNA towards treatment with polyamide is enhanced by pretreatment with the Chk2-I (Sigma). 1 μM NV1042 is not effective at preventing the cytopathic effect (CPE) and cell loss following 2 weeks of infection but inhibition of Chk2 kinase with Chk2 inhibitor dramatically enhances polyamide antiviral and cell protective ability.

[00051] Figure 8: Loss of viral BKV copies in response to 4 different polyamides compared with toxicity of the same polyamides in RPTECs. No toxicity is exhibited by the 4 polyamides at the highest dose of 10μM, while their IC_{50} values are in the low nanomolar range. Cidofovir, on the other hand, an antiviral that has been used for treatment of PyVAN patients, exhibits toxicity and low potency against BKV.

[00052] Figure 9: Figure 9A and 9B illustrate various types of guanidinyl radicals, including different substitution patterns and tautomers, which may be present in the polyamides of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[00053] The present invention provides compounds and methods for treating infections caused by double-stranded DNA viruses and other diseases in subjects/patients by administering polyamides and analogs of polyamide polymers.

[00054] For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in “Organic Chemistry”, Thomas Sorrell, University Science Books, Sausalito: 1999, and “March’s Advanced Organic Chemistry”, 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001.

[00055] The polyamides of the present invention may be generally described as polymeric or oligomeric molecules containing a plurality of carboxamide repeating units

such as those represented in the figure and, optionally, at least one guanidinyl radical per molecule.

[00056] Polyamides may be produced from known starting materials by known methods. See for example WO 05/033282, Belitsky et al., (2002) Bioorg. Med. Chem., 10, 2767-74; Zhang, et al. (2006) J. Am. Chem. Soc. 128:8766-76; Turner, et al. (2001) Organic Letters, 3:1201-03. Polyamides can be prepared using manual solid-phase synthesis as well as automated solid-phase chemistry. Each coupling is followed by HPLC and HPLC/mass spectrometry (HPLC/MS). Electrospray ionization and analysis of multiply-charged ions has allowed HPLC/MS to work effectively with a single quadrupole detector, but more sophisticated instruments are used for high resolution mass spectrometry (HRMS).

[00057] The polyamide compounds of the invention are made by standard solid phase Boc methods (Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* 1996, *118*, 6141) with Boc-β-PAM resin from Peptides International. However, the chemistry is altered to improve yields and purity as follows: PyBOP (benzotriazol-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate) is used as the coupling agent and extensive use is made of dimer building blocks to cut down the number of solid phase steps and to eliminate some byproducts. Purification is by reverse phase HPLC with 0.1% trifluoroacetic acid in the aqueous phase, except when formate salts are to be isolated, in which case formic acid is used in the aqueous mobile phase. Regarding stoichiometry, the highly basic TMG and Guan groups are always protonated upon isolation, as are all weakly to moderately basic nitrogens, given the acidity of the mobile phase.

[00058] With regard to chemical background, certain oligomers of nitrogen heterocycles can be used to bind to particular regions of double stranded DNA. Particularly, N-methyl imidazole (I), des-amino-N-methyl imidazole (Im), and N-methyl pyrrole (P) have a specific affinity for particular bases. This specificity can be modified based upon the order in which these compounds are linked. It has been shown that there is specificity

in that G/C is complemented by Im/P or I/P, C/G is complemented by P/Im or P/I, and A/T and T/A are redundantly complemented by P/P.

[00059] In effect, N-methyl imidazole and des-amino-N-methyl imidazole tend to be associated with guanine, while N-methyl pyrrole is associated with cytosine, adenine and thymine. By providing for two chains of the heterocycles, as 1 or 2 molecules, a 2:1 complex with double stranded DNA is formed, with the two chains; of the oligomer antiparallel, where G/C pairs have Im/P or I/P in juxtaposition, C/G pairs have P/Im or P/I, and T/A pairs have P/P in juxtaposition. The heterocycle oligomers are joined by amide (carbamyl) groups, where the NH may participate in hydrogen bonding with nitrogen unpaired electrons, particularly of adenine.

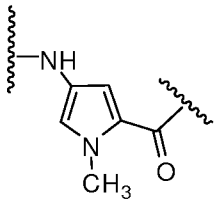
[00060] Polyamides may be synthesized to form hairpin compounds by incorporating compounds, such as gamma-aminobutyric acid (γ or $-\gamma-$) or gamma- 2,4-diaminobutyric acid (γ_{NH2} or $-\gamma_{NH2}-$), usually in the (R) form, to allow a single polyamide to form a complex with DNA.

[00061] Beta-alanine (β) may be substituted for a pair of N-methyl pyrrole groups when an AT or TA base pair is the target sequence. The added flexibility of the beta-alanine can help the entire polyamide stay "in register" with the target sequence of DNA.

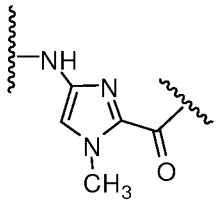
[00062] In some embodiments, the polyamide molecule begins with des- amino-N-methyl imidazole which has a specific affinity for guanosine. In other embodiments, the polyamide molecule ends with either 3-(Dimethylamino) propylamine (Da) or 3,3'-Diamino-N- methyldipropylamine (Ta). Dye molecules can be incorporated at the amino groups of the γ-amino-butyric acid, the Ta, or at both of these sites if both are available in the same molecule.

[00063] The polyamide building blocks are shown as radicals present in a polyamide chain rather than as free amino acid molecules or N-protected amino acids prior to their coupling into a polyamide. Pyrrole (Structure I below, typically abbreviated Py or P)

binds to the three nucleotides that present hydrogen bond acceptors in the minor groove, or A, T and C (21, 22). These nucleotides present only hydrogen bond acceptors to the minor groove: A and C each offer one lone pair of electrons while T offers two lone pairs from the carbonyl oxygen bound to C2. It is the amide NH of the hairpin pyrrole amino acids that is the hydrogen bond donor. So, the pyrrole ring acts as a curved spacer that presents amide NHs at the correct distance and curvature to match up with the pattern of hydrogen bond acceptors presented by A,C and T when located in B-form DNA. Imidazole (Structure II below) is typically abbreviated I or Im.

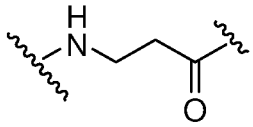


I. (P or Py)



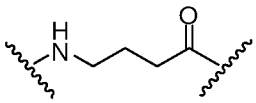
II. (I or Im)

[00064] Additional polyamide building blocks and binding rules can be found in the art (16, 23). However, these studies showed that β-alanine (Structure III (β), below) can act as an H-bond donor that is selective for A, T and C.



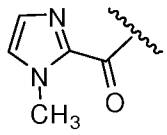
III (β)

[00065] For additional recognition, the γ-amino butyric acid (Structure IV (γ) below) building block used to form the hairpin turn was originally reported to bind A/T or TA, but not G/C or C/G base pairs. Similar preference for A/T over G/C base pairs is reported for the positively-charged amino tail that is present in most polyamides. This mimics the cationic group in distamycin(16, 19, 23-26). Standard hairpin polyamides often show the highest affinity for sequences that begin 5'-WWG-3', where W= A or T.

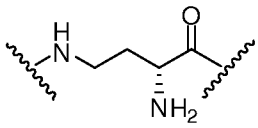


IV (γ or $-\gamma-$)

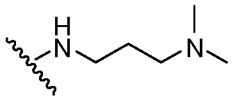
[00066] Other building blocks include, without limitation, desamino-imidazole (Formula V (Im)), (R)-2,4-diaminobutyric acid (Formula VI, γNH_2), the N-acetyl version of formula VI (γ_{NHAc} or $-\gamma_{NHAc}-$, not shown), 3-(dimethylamino)propylamine (Formula VII, Dp) and 3, 3'-diamino-N-methyldipropylamine (formula VIII, Ta).



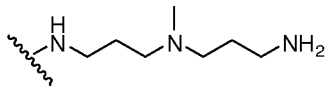
V (Im, dIm or desIm)



VI (γ_{NH2} or $-\gamma_{NH2}-$)

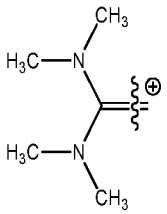


VII (Dp)

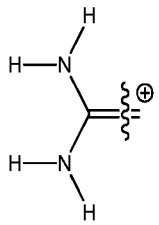


VIII (Ta)

[00067] New N-termini for polyamides that show high efficacy are the tetramethylguanidinyl (Structure IX) and unsubstituted guanidinyl (Structure X) groups, where structure X occurs in a number of tautomeric forms:



IX (TMG)



X (Guan)

[00068] The polyamides of the present invention may be generally described as polymeric or oligomeric molecules containing a plurality of carboxamide repeating units and at least one guanidinyl radical per molecule. In one embodiment, the polyamide is a compound having a polyamide backbone containing an interior unit selected from γ-

aminobutyric acid (γ); 2,4-diaminobutyric acid (γ_{NH2}), which may be either the (R) or (S) isomer and which may be linked in to the backbone of the polyamide through either the 2-amino group (to form an alpha turn) or through the 4-amino group (to form a gamma turn); or $H_2N(CH_2)_2CH(NHC(=O)NHR)CO_2H$ (either the (R) or (S) isomer), wherein R is $--(CH_2)_3--N(CH_3)--(CH_2)_3--NH_2$ ($\gamma_{NHR'}$) or $--(CH_2)_3--N(CH_3)_2$ ($\gamma_{NHR''}$), and at least one guanidinyl radical pendant to 2,4-diaminobutyric acid (γ_{NH2}), and/or pendant to $H_2N(CH_2)_2CH(NHC(=O)NHR)CO_2H$, wherein R is $--(CH_2)_3--N(CH3)--(CH_2)_3--NH_2$ ($\gamma_{NHR'}$), and/or at a terminal position of the polyamide backbone. The compound may be a pharmaceutically acceptable salt of such a polyamide. In the context of this invention, "interior" means at a position along the polymer backbone other than the terminal (end) positions or immediately adjacent to the terminal positions. The polyamide backbone may, in addition to the aforementioned interior unit, contain a plurality of units (for example, 5 to 30, or 7 to 28, or 9 to 24, or 11 to 22 or 15 to 21 or 16 to 21 units) selected from the group consisting of 4-amino-2-carbonyl-N-methylimidazole (Im), 4-amino-2-carbonyl-N-methylpyrrole (Py) and β-alanine (B).

[00069] In other aspects of the invention, the guanidinyl radical may be unsubstituted or substituted. That is, the three nitrogen atoms present in the guanidinyl radical may bear substituents other than hydrogen. Such substituents may be, for example, alkyl, aralkyl and/or aryl groups. Examples of these variously substituted guanidinyl radicals and their related tautomers are shown in Figure 9A and 9B. In one embodiment of the invention, two of the nitrogen atoms each bear two alkyl groups, such as C1-C4 alkyl groups. For example, the guanidinyl radical may be tetramethylguanidinyl (TMG, IX).

[00070] In one aspect of the invention, the guanidinyl radical is connected to a terminal 4-amino-2-carbonyl-N-methylpyrrole (Py) unit (i.e., the primary amine group initially present in the Py unit becomes part of the guanidinyl radical). In another aspect of the invention, a des-aminoimidazole (des-Im) forms the amino-terminus of the molecule and a guanidinyl radical is attached to an amino group elsewhere in the molecule, on for example the Ta or γ-NH_2 group.

[00071] The compound may contain a C terminus end group selected from 3,3'-diamino-N-methyldipropylamine (Ta) or 3-(dimethylamino)propylamine (Dp).

[00072] Compounds and formulations for treating PyV and/or HPV infections are described herein. According to this invention an “effective amount” of the compound or pharmaceutically acceptable composition is that amount effective for treating or lessening the severity of PyV and/or HPV infections.

[00073] If other indications are being treated with the polyamides described here, then an "effective amount" would be defined as per the norms of treatment for those diseases.

[00074] The method of treating PyV and/or HPV infections comprises administering a pharmaceutical composition comprising a polyamide of the invention.

[00075] Specific embodiments disclosed herein may be further limited in the claims using consisting of or and consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term "consisting of" excludes any element, step, or ingredient not specified in the claims. The transition term "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[00076] The pharmaceutical compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for treating or lessening the severity of PyV and HPV related disease. Ex-vivo organ or tissue such as kidney or bone marrow, treated with active polyamide prior to transplant surgery, can eliminate the reservoir of PyV and/or HPV (e.g., even if HPV is undetected in the ex-vivo organ or tissue, this is a possible precautionary measure to remove any HPV reservoir).

[00077] The invention relates to treating PyV or HPV infected entities including PyV or HPV in a biological sample or a patient (e.g., *in vitro* or *in vivo*), which method comprises administering to the patient (human or other animal), or contacting said biological sample with a pharmaceutical composition comprising a polyamide as described herein. The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof. The term "patient" includes animals, including mammals, humans, primates, dogs, cats, horses, pigs, cows, sheep and the like.

[00078] After the cells of an individual become exposed and infected with a PyV or HPV, a number of viral episome copies may become established within an infected cell. The PyV or HPV episomes further replicate as the cells divide. Polyamides designed to target A/T-rich regions of DNA efficaciously promote the clearance of viral episomes in the cases described before (HPV16, 18, 31). Efficacy is determined experimentally on a case by case basis. The methods of the present invention can be used beneficially as a therapeutic method to treat PyV or HPV infections.

[00079] The polyamides used to treat PyV or HPV include, without limitation, those described herein. In an aspect, the virus may be a PyV such as BKV, McPyV, or JCV.

[00080] In an embodiment, the invention provides a method of treating PyV and/or HPV affected cells comprising contacting the cells with a compound described herein. In an aspect of the invention, the method further comprises contacting the cells with an anti-viral agent. The anti-viral agent may be an Interferon, pegylated Interferon, cidofovir, CMX-001, leflunomide, adefovir, entecavir, lamivudine, telbivudine, tenofovir, acyclovir and/or other herpesvirus/cytomegalovirus drugs.

[00081] In an embodiment, the invention provides a method of treating PyV or HPV affected cells in a patient or subject, comprising administering to a patient or subject a

polyamide compound or pharmaceutical composition comprising a polyamide compound described herein.

[00082] In an embodiment, the polyamides and pharmaceutical compositions comprising polyamides used to treat double-stranded DNA virus-infected cells, for example, HPV infected cells or PyV infected cells, have the structure selected from the group of compounds listed in Table 1 and Table 3.

[00083] Polyamide oligomers may be synthesized starting with Boc-β-alanine-PAM solid phase synthesis resin, or a similar commercially available resin such as Fmoc-β-alanine-Wang resin, adding building blocks as required for the target sequence. The final step in the preparation of a guanidinylated polyamide is exemplified by incorporation of a tetramethylguanidinyl (TMG) group at the N-terminus TMG-polyamide synthesis involves placement of the tetramethylguanidinyl radical using HATU (2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate).

[00084] Table 1 lists a number of exemplary polyamides synthesized in accordance with the present invention. The HPLC/MW values given in Table 2 were obtained using low resolution high pressure liquid chromatography/mass spectrometry (LR HPLC/MS), which provides moderate precision masses of mixed isotopomers rather than average molecular weights or exact masses.

Table 1. Polyamide Compounds of the Invention

NV1002	ImPPPβPP-γ-PPPβPPPβDp
NV1003	ImPPPβPPβPPP-γ_{NH2}-PPPβPPPβPPPβDp
NV1004	ImPPPβPPβPPP-γ-PPPβPPPβPPPβDp
NV1005	ImPPPP-γ-PPPPPβTa
NV1006	ImPPPP-γ_{NH2}-PPPPPβTa
NV1007***	ImPPPP-γ-PPPPPβTa-FITC
NV1008***	ImPPPP-γ-PPPPPβTa-BOFLX
NV1009***	ImPPPP-γ_{NH2}-PPPPPβTa-FITC
NV1010	ImPPPP-γ-PPPPPβDp
NV1011	ImPPIm-γ-PPPPβDp
NV1012	ImImImP-γ-ImPPPβDp

NV1013	ImPPP-γ-PPImPβDp
NV1014	ImβImP-γ-ImPPPβDp
NV1015	ImβImP-γ-PPPPβDp
NV1016	ImβImImP-γ-PPPPPβDp
NV1017	ImPPIm-γ-PImImPβDp
NV1018	ImβImPP-γ-ImPPImPβDp
NV1019	ImPPImP-γ-ImPImPPβDp
NV1020	ImPPβPPP-γ_{NH2}-PPPβPPPβTa·4TFA
NV1021	ImImPIm-γ-PImPPβDp
NV1022	ImPPPβPPβ-γ-PPβPPPβPβDp
NV1023	ImPPβPPP-γ-PPβPPPPβDp
NV1024	ImPPβPPP-γ_{NH2}-PPβPPPPβDp
NV1025	ImPPPβPPP-γ-PPPβPPPββDp
NV1026	ImPPPβPPP-γ_{NH2}-PPPβPPPββDp
NV1027	ImPPPβPPβ-γ-PPβPPPβPβTa
NV1028	ImPPβPPP-γ-PPβPPPPβTa
NV1029	ImPPPβPPβ-γ_{NH2}-PPβPPPβPβDp
NV1030	ImPPβPPP-γ_{NH2}-PPβPPPPβTa
NV1031	ImPPPβPPP-γ-PPPβPPPββTa
NV1032	ImPPPβPPβ-γ_{NH2}-PPβPPPβPβTa
NV1033	ImPPPβPPP-γ_{NH2}-PPPβPPPββTa
NV1037	ImPPβPImPβPP-γ_{NH2}-PPβPPPβPPPβTa
NV1038	OHC-PPPPβDp
NV1039	OHC-PPPPPPβDp
NV1040	ImPPβPPImβPP-γ-PPβPPPβPPPβDp
NV1041	ImPPβPImPβPPIm-γ-βPβPPPβPPPβDp
NV1042	ImPPβPPImβPP-γ-PPβPPPβPPPβTa
NV1043	ImPPPIm-γ-βPPPPβDp
NV1044	ImPPPIm-γ-βPPPPβTa
NV1045	ImPPβPImPβPPIm-γ-βPβPPPβPPPβTa
NV1046	ImPβPPβPPImβPPP-γ-PβPPβPPPβPPPPβTa
NV1047	ImPPPβPPPβPP-γ-PPPβPPPβPPPβDp
NV1048	ImPPPβPPPβPP-γ-PPPβPPPβPPPβTa
NV1049	ImPPβPPImβPPαPPβPPPβPPPβTa (5 TFA)
NV1050	ImPPβPPImβPPα $_{NH2}$PPβPPPβPPPβDp (4 TFA)
NV1051**	ImPPβPPImβPPα$_{NHAc}$PPβPPPβPPPβTa (4 TFA)
NV1052**	ImPPPImα $_{NH2}$βPPPPβTa (5 TFA)
NV1053**	ImPPβPPPα$_{NHAc}$PPβPPPPβTa (3 TFA)
NV1054	Ac-βPPP-γ-PPβPPPPβTa
NV1055	Ac-PβPPP-γ-PPβPPPPβTa
NV1056	ImPPPβPPβ-γ-PPβPPPβPPDp
NV1057#	ImPPβPPP-γ-PPβPPPPβdesTa (desmethyl Ta)
NV1058	ImPPPIm-γ_{NH2}-βPPPPβTa
NV1059	ImPPPIm-γ_{NHAc}-βPPPPβTa

NV1060	ImPPPIm-γ_{NHAc}-βPPPPβDp
NV1061	ImPPβPPP-γ_{NH2}-PPβPPPPβTa
NV1062	ImPPβPPP-γ_{NHAc}-PPβPPPPβTa
NV1063	ImPPβPPP-γ_{NHAc}-PPβPPPPβDp
NV1064	ImPPβPPP-γ_{NH2}-PPβPPPPβDp
NV1065	ImPPPIm-γ_{NH2}-βPPPPβDp
NV1066	ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβDp
NV1067	ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβTa
NV1068	ImPPβPPImβPP-γ_{NHAc}-PPβPPPβPPPβDp
NV1069	ImPPβPPP-γ-PPβPPPPPTa • 3TFA
NV1070	ImPPβPPImβPP-γ-PPβPPPβPPPPTa • 4TFA
NV1071	TMG -PPβPPβPIm-γ_{NH2}-PβPPβPPPβPPTa • 5TFA
NV1072	TMG -PPPβPPβPIm-γ_{NHR}-PβPPβPPPβPβTa • 6TFA
NV1073	TMG -PPPβPPβPIm-γ_{NH2}-PβPPβPPPβPβTa • 5TFA
NV1074	TMG -PPPβPPβP-γ_{NHR}-PPPβPPPβPβTa • 5TFA
NV1075	TMG -PPPβPPβPIm-γ_{NH2}-PβPPβPPPβPβDp • 4TFA
NV1076	TMG -PPPβPPβP-γ_{NH2}-PPPβPPPβPβTa
NV1077	TMG -PPPβPPβP-γ_{NH2}-PPPβPPPβPβDp
NV1078	TMG –PβPPImβPP-γ-PPβPPPβPPPβTa • 4TFA
NV1079	TMG -PPPβPPβP-$\gamma_{NHR'}$-PPPβPPPβPβDp • 3TFA
NV1080	TMG –PβPPImβPP-γ-PPβPPPβPPPβDp • 3TFA
NV1081	TMG –PβPPP-γ-PPβPPPPβDp • 2TFA
NV1082	TMG –PβPPP-γ-PPβPPPPβTa • 3TFA
NV1083	TMG –PPβPPP-γ-PPβPPPPβDp • 2TFA
NV1084	TMG –PPβPPP-γ-PPβPPPPβTa • 3TFA
NV1085	TMG –PPβPPImβPP-γ-PPβPPPβPPPβTa
NV1086	TMG –PPβPPImβPP-γ-PPβPPPβPPPβDp
NV1087	TMG –PPPβPPβPIm-γ-PβPPβPPPβPβTa
NV1088	TMG –PPPβPPβPIm-γ-PβPPβPPPβPβDp
NV1089	TMG –PpβPPβPIm-γ-PβPPβPPPβPβDp
NV1090	TMG –PpβPPβPIm-γ-PβPPβPPPβPβTa
NV1092	ImPPβPP-γ-PPβPPPPβTa
NV1093	ImPPβPPImβP-γ-PPβPPPβPPPβTa
NV1094	TMG –PImβP-γ-PPPβPPPβTa
NV1095	TMG –PPβPPβP-γ_{NH2}-PPPβPPPβPβTa
NV1096	TMG –PpβPPβPIm-γ_{NH2}-PβPPβPPPβPβTa
NV1097	TMG –PPβPPβP-γ-PPPβPPPβPβ-Ta • 3TFA
NV1098	TMG –PPPβPPβP-γ-PPPβPPPβPβ-Ta • 3TFA
NV1101	TMG –PyPyPyβPyPyβPyIm-γ-PyβPyPyβPyPyPyβPyβ-Ta-FAM (3 TFA)
NV1102	TMG –PImPIm-γ-PPPPβTa
NV1103	TMG –PImβIm-γ-PβPPβTa
NV1104	TMG –PImPIm-γ-PβPPβTa

NV1105	TMG –PImβIm-γ-PPPPβTa
NV1106	Guan-PImβImPβPPβTa
NV1107	Guan-PPβPPImβPP-γ-PPβPPPβPPPβTa (4 TFA)
NV1108	Guan-PPβPPP-γ-PPβPPPPβTa (3 TFA)
NV1109	Guan-PPβPPImβPP-γ-PPβPPPβPPPβDp (3 TFA)
NV1110	Guan-PPβPPP-γ-PPβPPPPβDp (2 TFA)
NV1111	Guan-PPPβPPβPIm-γ-PβPPβPPPβPβTa (4 TFA)
NV1112	Guan-PPPβPPβPIm-γ-PβPPβPPPβPβDp (3 TFA)
NV1113	TMG –IPPβPPP-γ-PPβPPPPβTa (4 TFA)
NV1114	TMG –IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA)
NV1115	Guan-IPPβPPP-γ-PPβPPPPβTa (4 TFA)
NV1116	Guan-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA)
NV1117	IPPβPPP-γ-PPβPPPPβTa-BODIPY-FLX (2 TFA)
NV1118	Ac-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA)
NV1119	PPβPPβPIm-γ-PβPPβPPPβPβTa
CHC2001	ImPPβPPP-γ-PPβPPPPβTa (3 HCO_2H)
CHC2002	ImPPPβPP-γ-PPPβPPPβTa (3 HCO_2H)
KJK6045f26-28	TMG –ImPβPPP-γ-PβPPPPβTa (4 HCO_2H)
KJK6047-1	Guan-ImPβPPP-γ-PβPPPPβTa (4 HCO_2H)
KJK6048	ImPβPPP-γ-PβPPPPβTa (3 HCO_2H)
KJK6049	ImPPβPP-γ-PPβPPPβTa (3 HCO_2H)
KJK6050f16-18	TMG –ImPPβPP-γ-PPβPPPβTa (4 HCO_2H)
KJK6062	Guan-ImPPβPP-γ-PPβPPPβTa (4 HCO_2H)
KJK6065f17-19	TMG –PPImβPP-γ-PPβPPPβTa (4 HCO_2H)
KJK6067f12	Guan-PPImβPP-γ-PPβPPPβTa (4 HCO_2H)
KJK6068f20-22	PPImβPP-γ-PPβPPPβTa (3 HCO_2H)
KJK6076	ImPPβPPImβPP-γ-PPβPPPβPPPβTa (4 HCO_2H)
KJK6099	ImPPPβPP-γ-PPPβPPPβDp (2 TFA)
FT1138	ImPPβPPImβPP-γ-PPβPPPβPPPβTa-AF488 (HCO_2H)
FT1139	ImImPIm-γ-PβPPβTa-AF488 (2 HCO_2H)

[00085] In accordance with the foregoing:
GUAN = guanidinyl radical;
TMG = tetramethylguanidinyl;
P = 4-amino-2-carbonyl-N-methylpyrrole;
γ = gamma-aminobutyric acid;
γ_{NH2} = (R)-2,4-diaminobutyric acid reacted through either the 2-amino group or the 4-amino group;
γ_{NHAc} = (R)-2-(acetylamino)-4-aminobutyric acid;
β = beta-alanine;
Im = 4-amino-2-carbonyl-N-methylimidazole;

Ta = 3,3'-diamino-N-methyldipropylamine;
Dp = (dimethylamino)propylamine;
TFA = trifluoroacetic acid;
HCO_2H = formate;
AF488- AlexaFluor-488 fluorophore; and
α means that the γ-aminobutyric acid formed an α-linked hairpin rather than the typical γ-linked hairpin; and AF488= AlexaFluor-488 fluorophore

[00086] In an embodiment, the guanidinyl radical is unsubstituted (i.e., the nitrogen atoms in the guanidinyl radical do not bear any substituents other than hydrogen). In an embodiment, the guanidinyl radical is monosubstituted or gem-disubstituted, or is N,N'-disubstituted, N,N,N'-trisubstituted, or N,N,N',N'-tetrasubstituted. In a further embodiment, a guanidinylated polyamide in accordance with the invention may be N,N',N'-trisubstituted.

[00087] Figure 9A and 9B illustrate various types of guanidinyl radicals, including different substitution patterns and tautomers, which may be present in the polyamides of the present invention.

Table 2. Polyamide compounds used for evaluating anti-polyomavirus activity and/or anti-papillomavirus activity.

Compound	Molecular formula of free base	calc. exact mass M	calc. avg. MW	HPLC/MW (ESI^+)	HRMS
NV1071	$C_{114}H_{145}N_{41}O_{20}$	2408.159	2409.63	2409.8 $[M+H]+$ 1205.5 $[M+2H]^{2+}$	2408.14725 $[M]^+$
NV1072	$C_{125}H_{167}N_{45}O_{22}$	2650.3332	2651.95	1326.5 $[M+2H]^{2+}$	2650.31905 $[M]^+$
NV1073	$C_{117}H_{150}N_{42}O_{21}$	2479.1961	2480.71	2481.0 $[M+H]^+$ 1241.0 $[M+2H]^{2+}$	2479.18193 $[M]^+$
NV1074	$C_{117}H_{157}N_{41}O_{20}$	2456.2529	2457.76	2458.2 $[M+H]^+$ 1229.5 $[M+2H]^{2+}$	2456.24158 $[M]^+$
NV1075	$C_{115}H_{145}N_{41}O_{21}$	2436.1539	2437.64	2438.2 $[M+H]^+$ 1219.5 $[M+2H]^{2+}$	2436.14773 $[M]^+$
NV1076	$C_{109}H_{140}N_{38}O_{19}$	2285.1157	2286.52	2287.0 $[M+H]^+$ 1144.0 $[M+2H]^{2+}$	2285.10297 $[M]^+$
NV1077	$C_{107}H_{135}N_{37}O_{19}$	2242.0735	2243.45	2244.0 $[M+H]^+$ 1122.3 $[M+2H]^{2+}$	2242.0638 $[M]^+$
NV1078	$C_{114}H_{144}N_{40}O_{20}$	2393.1481	2394.62	2395.0 $[M+H]^+$ 1198.0 $[M+2H]^{2+}$	2393.13581 $[M]^+$
NV1079	$C_{113}H_{147}N_{39}O_{20}$	2370.1685	2371.63	2372.0 $[M+H]^+$ 1186.5 $[M+2H]^{2+}$	2370.15878 $[M]^+$
NV1080	$C_{112}H_{139}N_{39}O_{20}$	2350.1059	2351.55	2352.0 $[M+H]^+$ 1176.5 $[M+2H]^{2+}$	2350.09462 $[M]^+$
NV1081	$C_{83}H_{106}N_{28}O_{14}$	1718.8443	1719.91	1720.5 $[M+H]^+$ 860.5 $[M+2H]^{2+}$	1718.834 $[M]^+$
NV1082	$C_{85}H_{111}N_{29}O_{14}$	1761.8865	1762.98	1763.5 $[M+H]^+$ 882.0 $[M+2H]^{2+}$	1761.8757 $[M]^+$
NV1083	$C_{89}H_{112}N_{30}O_{15}$	1840.8923	1842.03	1842.5 $[M+H]^+$ 921.5 $[M+2H]^{2+}$	1840.88244 $[M]^+$
NV1084	$C_{91}H_{117}N_{31}O_{15}$	1883.9345	1885.1	1885.5 $[M+H]^+$ 943.0 $[M+2H]^{2+}$	1883.92375 $[M]^+$

Compound	Molecular formula of free base	calc. exact mass M	calc. avg. MW	HPLC/MW (ESI^+)	HRMS
NV1085	$C_{120}H_{150}N_{42}O_{21}$	2515.1961	2516.74	2517.0 $[M+H]^+$ 1259.0 $[M+2H]^{2+}$	2515.18393 $[M]^+$
NV1086	$C_{118}H_{145}N_{41}O_{21}$	2472.1539	2473.68	2474.0 $[M+H]^+$ 1237.5 $[M+2H]^{2+}$	2472.14515 $[M]^+$
NV1087	$C_{117}H_{149}N_{41}O_{21}$	2464.1852	2465.7	2466.0 $[M+H]^+$ 1233.5 $[M+2H]^{2+}$	2464.1686 $[M]^+$
NV1088	$C_{115}H_{144}N_{40}O_{21}$	2421.143	2422.63	2423.0 $[M+H]^+$ 1212.0 $[M+2H]^{2+}$	2421.12661 $[M]^+$
NV1089	$C_{109}H_{138}N_{38}O_{20}$	2299.095	2300.5	2300.8 $[M+H]^+$ 1151.0 $[M+2H]^{2+}$	2299.116 $[M]^+$
NV1090	$C_{111}H_{143}N_{39}O_{20}$	2342.1372	2343.57	2343.8 $[M+H]^+$ 1172.5 $[M+2H]^{2+}$	2342.15 $[M]^+$
NV1094	$C_{84}H_{110}N_{30}O_{14}$	1762.8818	1763.96	1763.8 $[M+H]^+$ 882.5 $[M+2H]^{2+}$	1762.8907 $[M]^+$
NV1095	$C_{103}H_{134}N_{36}O_{18}$	2163.0677	2164.4	1083.0 $[M+2H]^{2+}$	
NV1096	$C_{111}H_{144}N_{40}O_{20}$	2357.1481	2358.59	1180.0 $[M+2H]^{2+}$	2358.17183 $[M+H]^+$
NV1097	$C_{103}H_{133}N_{35}O_{18}$	2148.0574	2149.39	2149.8 $[M+H]^+$ 1075.5 $[M+2H]^{2+}$	2148.051 $[M]^+$
NV1098	$C_{109}H_{139}N_{37}O_{19}$	2270.1054	2271.51	2271.8 $[M+H]^+$ 1136.5 $[M+2H]^{2+}$	
NV1101	$C_{138}H_{160}N_{41}O_{27}$	2823.2407	2825.01	1412.5 $[M+2H]^{2+}$	
NV1102	$C_{65}H_{87}N_{25}O_{10}$	1377.70716	1378.56	1378.6 $[M+H]^+$ 689.8 $[M+2H]^{2+}$	1377.7012
NV1103	$C_{59}H_{85}N_{23}O_{10}$	1275.6801	1276.46	1276.6 $[M+H]^+$ 638.8 $[M+2H]^{2+}$	1275.6801
NV1104	$C_{62}H_{86}N_{24}O_{10}$	1326.69626	1327.51	1327.6 $[M+H]^+$ 664.4 $[M+2H]^{2+}$	1326.6897

Compound	Molecular formula of free base	calc. exact mass M	calc. avg. MW	HPLC/MW (ESI+)	HRMS
NV1105	$C_{62}H_{86}N_{24}O_{10}$	1326.69626	1327.51	1327.6 $[M+H]^+$ 664.4 $[M+2H]^{2+}$	1326.69
NV1106	$C_{55}H_{77}N_{23}O_{10}$	1219.6227	1220.35	1220.4 $[M+H]^+$ 610.8 $[M+2H]^{2+}$	2408.14725 $[M]^+$

[00088] Table 3. Polyamide sequences and HRMS for compounds used for evaluating anti-polyomavirus activity. MS values listed represent M+ unless noted otherwise. TMG- = tetramethylguanidinyl, Guan- = guanidinyl, Ac = N-acetyl, OHC = N-formyl, R = -CO-$(CH_2)_3$-N(CH_3)-$(CH_2)_3$-NH_2, R' = -CO-$(CH_2)_3$-N$(CH_3)_2$; other building blocks are defined above.

Polyamide #	Polyamide Sequence	Theor. MS	Actual MS
NV1002	ImPPPβPP–γ–PPPβPPPβDp	1850.8403	1850.8408
NV1021	ImImPIm–γ–PImPPβDp	1223.5597	1223.5611
NV1028	ImPPβPPP–γ–PPβPPPPβTa	MH+ = 1894.8908	1894.8933
NV1029	ImPPPβPPβ–γ_{NH2}–PPβPPPβPβDp	2007.9254	2007.9282
NV1030	ImPPβPPP–γ_{NH2}–PPβPPPPβTa	MH+ = 1909.9018	1910.9064
NV1039	OHC-PPPPPPβDp	933.4358	933.4371
NV1042	ImPPβPPImβPP–γ–PPβPPPβPPPβTa	MH+ = 2526.1447	2526.1557
NV1054	Ac-βPPP–γ–PPβPPPPβTa	MH+ = 1583.7647	1583.7709
NV1055	Ac-PβPPP–γ–PPβPPPPβTa	MH+ = 1705.8127	1705.8182
NV1056	ImPPPβPPβ–γ–PPβPPPβPPDp	2043.9254	2043.9283

NV1058	ImPPPIm–γ_{NH2}–βPPPPβTa	MH+ = 1473.7157	1473.7155
NV1059	ImPPPIm–γ_{NHAc}–βPPPPβTa	MH+ = 1515.7263	1515.7280
NV1060	ImPPPIm–γ_{NHAc}–βPPPPβDp	MH+ = 1472.6841	1472.6855
NV1065	ImPPPIm–γ_{NH2}–βPPPPβDp	MH+ = 1430.6735	1430.6738
NV1066	ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβDp	MH+ = 2497.1128	2497.1335
NV1067	ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβTa	2540.1550	2540.1783
NV1068	ImPPβPPImβPP–γ_{NHAc}–PPβPPPβPPPβDp	2539.1233	2539.1348
NV1070	ImPPβPPImβPP–γ–PPβPPPβPPPPTa	MH+ = 2577.1628	2577.1153
NV1071	TMG-PPβPPβPIm–γ_{NH2}–PβPPβPPPβPPTa	2408.1590	2408.1472
NV1072	TMG-PPPβPPβPIm–γ_{NHR}–PβPPβPPPβPβTa	2650.3332	2650.3190
NV1073	TMG-PPPβPPβPIm–γ_{NH2}–PβPPβPPPβPβTa	2479.1961	2479.1819
NV1078	TMG-PβPPImβPP–γ–PPβPPPβPPPβTa	2393.1481	2393.1358
NV1079	TMG-PPPβPPbP–$\gamma_{NHR'}$–PPPβPPPβPβDp	2370.1685	2370.1588
NV1080	TMG-PβPPImβPP–γ–PPβPPPβPPPβDp	2350.1059	2350.0946
NV1081	TMG-PβPPP–γ–PPβPPPPβDp	1718.8443	1718.8340
NV1082	TMG-PβPPP–γ–PPβPPPPβTa	1761.8865	1761.8757
NV1083	TMG-PPβPPP–γ–PPβPPPPβDp	1840.8923	1840.8824
NV1084	TMG-PPβPPP–γ–PPβPPPPβTa	1883.9345	1883.9238
NV1085	TMG-PPβPPImβPP–γ–PPβPPPβPPPβTa	2515.1961	2515.1839
NV1086	TMG-PPβPPImβPP–γ–PPβPPPβPPPβDp	2472.1539	2472.1452
NV1087	TMG-PPPβPPβPIm–γ–PβPPβPPPβPβTa	2464.1852	2464.1686
NV1088	TMG-PPPβPPβPIm–γ–PβPPβPPPβPβDp	2421.1430	2421.1266
NV1089	TMG-PPβPPβPIm–γ–PβPPβPPPβPβDp	2299.0950	2299.1160
NV1090	TMG-PPβPPβPIm–γ–PβPPβPPPβPβTA	2342.1372	2342.1500

NV1092	ImPPβPP–γ–PPβPPPPβTa	1771.83498	1771.8333
NV1093	ImPPβPPImβP–γ–PPβPPPβPPPβTa	2403.0967	2403.0924
NV1097	TMG-PPβPPβP–γ–PPPβPPPβPβTa	2148.0574	2148.0510
NV1098	TMG-PPPβPPβP–γ–PPPβPPPβPβ-Ta	2270.1048	2270.1099
NV1104	TMG-PImPIm–γ–PβPPβTa	1326.6963	1326.6897
NV1106	Guan-PImβIm–γ–PβPPβTa	1219.6223	1219.6190
NV1107	Guan-PPβPPImβPP–γ–PPβPPPβPPPβTa	2459.1334	2459.1157
NV1108	Guan-PPβPPP–γ–PPβPPPPβTa	1827.8719	1827.8608
NV1109	Guan-PPβPPImβPP–γ–PPβPPPβPPPβDp	2416.0912	2416.0769
NV1110	Guan-PPβPPP–γ–PPβPPPPβDp	1784.8297	1784.8185
NV1111	Guan-PPPβPPβPIm–γ–PβPPβPPPβPβTa	2408.1225	2408.1075
NV1112	Guan-PPPβPPβPIm–γ–PβPPβPPPβPβDp	2365.0803	2365.0657
NV1113	TMG-IPPβPPP–γ–PPβPPPPβTa	2006.9778	2006.9653
NV1114	TMG-IPPβPPIβPP–γ–PPβPPPβPPPβTa	2638.2393	2638.2232
NV1115	Guan-IPPβPPP–γ–PPβPPPPβTa	1950.9152	1950.9034
NV1116	Guan-IPPβPPIβPP–γ–PPβPPPβPPPβTa	2582.1767	2582.1607

[00089] Polyamide-DNA binding has been reported to interfere with protein-DNA binding, and polyamides have been reported to inhibit gene expression, presumably through competition with transcription factors for DNA binding sites.

[00090] The present invention provides methods for treating polyomavirus and/or papillomavirus infections and other diseases by administering polyamides and analogs of polyamide polymers.

[00091] Time-course experiments of the anti-PyV action of the polyamides of this invention lead to the discovery that a single dose of certain active molecules delivered

to polyomavirus-infected renal proximal tubule epithelial cells (RPTECs) on day 2 post-infection, causes a significant reduction in viral copy numbers as late as 11 days post-infection, as shown in Figure 6.

[00092] PyV establishes it's small, circular, chromatinized, supercoiled genomes in the nucleus of infected cells. Without being bound by theory, polyamides used in the present invention can bind the circular PyV and/or HPV genomes, causing changes within the viral episomes that are recognized and not tolerated by the host cells. Cellular mechanisms are then activated, resulting in the rapid loss and degradation of the episome. Thus, binding of polyamides to DNA activates a process resulting in specific elimination of viral rather than host DNA sequences.

[00093] The polyamides described herein provide a therapeutic agent to target PyV and/or HPV. Possible targets within the viruses may include sequences required for tethering, maintenance, or replication, or the polyamides may trigger cellular recognition of the viral DNA as foreign resulting in its clearance.

[00094] In still yet other embodiments, the polyamides used in combination with other antivirals, such as, without limitation, Interferon, cidofovir, CMX-001, leflunomide, adefovir, entecavir, lamivudine, telbivudine, or tenofovir, acyclovir and other Herpes/cytomegaloviral drugs, and anti-HIV drugs. The polyamides can also be used in combination with photodynamic therapy, radiation therapy and chemotherapy.

[00095] Administration of a "therapeutically effective amount" of a pharmaceutical composition comprising the polyamides of the invention results in treating or lessening the severity of PyV and papillomavirus infections in subjects infected with PyV or HPV. The therapeutically effective amount of a pharmaceutical composition comprises the active polyamides in a unit dosage form which is amenable to administration to a subject for the efficacious treatment of a viral infection. Such pharmaceutical compositions and unit dosage forms may facilitate patient compliancy and may comprise conventional or new ingredients in conventional or special proportions, with or

without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended dosage range to be employed for facile and efficacious treatment of viral infections.

[00096] The active pharmaceutical ingredients of the invention, together with one or more conventional adjuvants, carriers, or diluents, may be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids (e.g., coated or uncoated tablets or filled capsules), or liquids or semi-solids (e.g., solutions, suspensions, emulsions, gels, elixirs, or capsules filled with the same), for oral or mucosal use as well as injectables.

[00097] The term "carrier" applied to pharmaceutical compositions of the invention refers to a diluent, excipient, or vehicle with which an active compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water, saline solutions, aqueous dextrose solutions, aqueous glycerol solutions, and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, Dimethysulfoxide (DMSO) or D5W vehicle, and the like. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin, 18th Edition.

[00098] Polyamides can be in the form of pharmaceutically acceptable salts such as trifluoroacetate (TFA) salts, formate salts, as well as chloride, succinate, ascorbate salts and the like. They can also be formulated with excipients such as PEG-400, propylene glycol and the like.

[00099] To increase stability, the polyamide drug may be placed in aqueous solution with an antioxidant such as ascorbic acid, BHT or BHA in order to develop a more stable formula. (See Mayers C. L., et al. (1993) Pharma Res, 10: 445-448, and Stuhar M., (1984) Farmaceuticky Obzor, 53; 499-504).

[000100] For delivery to the vagina and cervix, polyamides may be formulated as solutions, emulsions, suspensions, tablets, gels, foams, suppositories, films, sponges and vaginal rings. Formulations include gels (e.g., gels prepared using gelling agents such as hydroxy ethyl cellulose and polyacrylic acids, e.g., cross-linked acrylic acid based polymers such as those sold under the brand name CARBOPOL®), and polyvinyl alcohol films that can be administered by an applicator to the target site. Alternatively, lower viscosity liquid formulations (e.g. Polyethylene glycol solutions) can be delivered in a polyurethane sponge to the area around the cervix. (Okada, (1991) in "Peptide and Protein Drug Delivery" V. H. Lee, ed., pp. 663-666, Marcel Dekker, NY; Garg, et al. (2001) Pharm. Tech. 25:14-24). Because of the polyamides' charge, the polyamides may be formulated in a controlled delivery vehicle by using carbomers (such as those sold under the brand name CARBOPOL®). If the polyamide has a charge of +1 or +2, by adjusting the ionic strength of the formulation one may bind the polyamide electrostatically to the carbomer and thereby control the release rate. In a semisolid dosage form, the release rate may be evaluated in a membrane apparatus as described in the US Pharmacopeia (Dipiano, et al., PCT International Publication No. WO 04/064913) for drug diffusion from semisolid dosage forms. Polyamides formulated in carbomer-based gels which exhibit significant yield stresses, and also have potential bioadhesive properties (Kieweg, et al. (2004) J. Pharm Sci. 93, 2941-52.

[000101] Any of the excipients used for commercial vaginal formulations (Garg et al., 2001) may be adapted for use with the polyamide compounds of the present invention. A number of commonly used excipients such as PEG (polyethylene glycol), PVA (polyvinyl alcohol) and Tween surfactants can also be employed. In addition to antioxidants, further compatibilizers or stabilizers may be used. Solid forms may allow for more stable formulas with a longer shelf life due to their physical state. Emulsions made from bioadhesives using polymers such as carbomers may be useful. HPMC (hydroxymethylpropyl cellulose), PVA and lipid complexes can be used with lower solubility drugs. Lipidic systems may then be suspended in a viscoelastic gel for delivery of the insoluble polyamide.

[000102] For more sustained or effective delivery, cervical barrier devices available such as diaphragms that can deliver the drug at the cervix site over many hours can be used for delivery that is even more continuous vaginal rings or slow release implantable polymer films can be employed. In addition, several new vaginal delivery systems in clinical testing such as vaginal sponge technology and the SILCS diaphragm, a single size silicone device that can deliver drug to both the cervix and vaginal wall (Cohen, (2004) The Microbiocide Quarterly, 2:15-19) may be used. For improved continuous delivery of the drug over an extended period, vaginal rings are available with slow release of the drug from the ring composite (Cohen, 2004; Hussain and Ahsan, (2005), J. Controlled Release 103:301-13). There are also numerous other applicators and formulas that have been developed for controlled vaginal drug delivery (Robinson (1999) Proc. Of the 26th Intl. Symp. Controlled Release of Bioactive Materials, 26:2-3; Hussain and Ahsan, 2005).

[000103] Formulations for transdermal delivery include lipid-based formulas for delivery of protein pharmaceuticals to genital warts (Foldvari et al., (1999), Biotech. Appl. Biochem. 30:129-37; Leigh (2003) Drugs and the Pharm. Sci., 126:791-800; Lee et al., (2004) Biomaterials, 26:205-10), bioadhesives formulations (Bogataj and Mrhar (1998) Bioadhesive mucosal drug delivery systems, 49:445-57; Amaral et al. (1999) Contraception, 60:361-66; Barry, (1987) in "Drug Delivery systems", Johnson and Lloyd-Jones, eds, Ch. 11, Ellis Horwood, Chichester; Vermani, et al. (2002) Drug Dev. Indust. Pharm. 28:1133-46) and novel polymer systems. The novel polymers include partially absorbable biodegradable antiviral intravaginal rings (Shalaby, (2005) U.S Patent Application Publication No. 2005/053639), bilaminar bioadhesive polymeric films applied directly to the cervix (Sidhu et al., (1997) Br. J. Obstetrics and Gynaecology, 104:145-49) novel, slow-release polymer discs at the cervical mucosa and thermogelling systems that have the advantage of potentially much greater bioadhesion and dosage form retention. (Saltzman and Radomsky (1990) Polymer Preprints, 31:245-46; Edelman and Mark (1998) Nature Biotech, 16:136-37). Polyamides may also be formulated using cell membrane penetrating peptides (Gupta, et al. (2005) Adv. Drug Del Rev. 57:637-51; Wadia and Dowdy (2005) Adv. Drug Del. Rev., 57:579-96.

[000104] The polyamides of the present invention can also be formulated with a pharmaceutically-acceptable polymer designed to shorten or lengthen time before renal clearance.

[000105] Polyamides in accordance with the present invention can also be formulated to deliver an aerosol treatment of the lungs, mouth or throat. Direct injection into HPV lesions may also be employed for external (cutaneous) or mucosal skin infections.

[000106] Other disease indications may require systemic treatment with the present polyamides, i.e., by injection, or additional, common or known drug delivery methods.

[000107] It will also be appreciated that certain compounds of the present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative or a prodrug thereof. According to the present invention, a pharmaceutically acceptable derivative or a prodrug includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a subject in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

[000108] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

[000109] Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J.

Pharmaceutical Sciences, 1977, 66, 1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, including trifluoroacetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}\,alkyl)_4$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[000110] As described above, the pharmaceutically acceptable compositions of the present invention comprise, in addition to one or more polyamide compounds, a pharmaceutically acceptable carrier, adjuvant, or vehicle, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension

aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this invention.

[000111] In an embodiment, the present invention provides polyamides and analogs of polyamides which are useful for treating polyomavirus infections. Table 4 presents a summary of measured IC_{50} values and IC_{90} values of certain polyamides against polyomaviruses.

Table 4. Activity of polyamides against SV40 and BKV

Polyamide #	SV40 (IC_{50})	SV40 (IC_{90})	BKV-Dun (IC_{50})	BKV-Dun (IC_{90})	BKV-TU (IC_{50})	BKV-TU (IC_{90})
NV1111	0.005	0.031	0.003	0.045	0.026	0.101
NV1071	0.006	0.156	0.003	0.049	0.006	0.017
NV1112	0.012	0.072	0.002	0.016	0.003	0.028
NV1078	0.017	0.308	0.012	0.084	0.016	0.093
NV1072	0.024	0.123	0.008	0.057	0.006	0.061
NV1042	0.032	0.161	0.009	0.063	0.010	0.057
NV1085	0.033	0.375	0.004	0.062	0.002	0.015
NV1088	0.034	0.097	0.002	0.023	0.002	0.014
NV1087	0.035	0.099	0.006	0.078	0.003	0.038
NV1107	0.036	0.557	0.002	0.013	0.012	0.147
NV1098	0.037	0.154	0.004	0.134	0.003	0.057
NV1070	0.040	0.136	0.055	0.323	0.068	0.214
NV1073	0.040	1.186	0.554	>10	0.117	1.129
NV1097	0.041	0.210	0.053	0.206	0.008	0.065
NV1116	0.044	0.165	0.001	0.066	0.008	0.052
NV1114	0.045	0.171	0.002	0.019	0.036	0.411
NV1066	0.045	0.173	0.020	0.189	0.014	0.167

NV1086	0.058	0.381	0.023	0.243	0.003	0.065
NV1079	0.062	0.325	0.014	0.173	0.011	0.080
NV1093	0.078	0.484	0.038	1.480	0.010	0.094
NV1067	0.093	0.176	0.555	3.057	0.019	0.186
NV1080	0.109	0.429	0.080	1.937	0.016	0.190
NV1109	0.117	0.468	0.004	0.078	0.053	0.832
NV1115	0.160	0.371	0.039	0.136	0.188	1.447
NV1028	0.218	0.735	0.437	10.000	0.062	1.517
NV1113	0.347	0.876	0.017	0.134	0.026	3.397
NV1092	0.415	0.781	1.021	>10	0.115	>10
NV1030	0.422	1.084	4.064	>10	0.167	0.962
NV1068	0.424	4.396	0.533	>10	0.041	>10
NV1108	0.451	1.284	0.060	1.035	0.148	1.174
NV1056	0.637	>10	0.514	10.000	1.635	>10
NV1084	1.000	>10	NA	NA	0.192	5.548
NV1110	1.746	9.400	0.103	1.659	0.119	>10
NV1082	2.372	7.349	NA	NA	3.394	9.728
NV1083	5.173	9.174	NA	NA	0.325	>10
NV1029	7.165	>11	NA	NA	NA	NA
NV1081	8.589	>10	NA	NA	NA	NA
NV1054	NA	NA	NA	NA	9.309	>10
NV1055	NA	NA	NA	NA	1.282	>10
NV1058	NA	NA	NA	NA	NA	NA
NV1059	NA	NA	NA	NA	NA	NA
NV1060	NA	NA	NA	NA	NA	NA
NV1065	NA	NA	NA	NA	NA	NA
NV1021	NA	NA	NA	NA	NA	NA
NV1039	NA	NA	NA	NA	NA	NA
NV1104	NA	NA	NA	NA	NA	NA
NV1106	NA	NA	NA	NA	NA	NA
NV1002	NA	NA	NA	NA	5.668	>10
NV1089	not tested	not tested	0.007	0.266	0.003	0.051
NV1090	not tested	not tested	0.003	0.053	0.002	0.016

[000112] In Table 4, "NA" indicates no measurable antiviral response was obtained relative to control at the highest dose tested (10 μM). The IC_{50} is the concentration of compound required for 50% decrease of viral DNA concentration *in vitro*. The IC_{90} is the concentration of compound required for 90% decrease in viral DNA concentration *in vitro*.

[000113] As shown in the figures, a single polyamide treatment caused a profound decrease of viral DNA levels for the period of infection relative to the untreated cells so

that by day 12 (11 days after polyamide treatment) only 25% of the viral DNA levels found in the untreated samples was present in the treated samples.

[000114] One skilled in the art may conclude that the polyamides of the invention would provide therapeutic efficacy in clinically relevant polyomaviruses.

[000115] In an embodiment, the present invention provides polyamides and analogs of polyamides that are useful for treating HPV infections and other diseases.

[000116] Time-course experiments of the anti-HPV action of the polyamides of this invention led to the discovery that certain active molecules decrease HPV DNA levels in human keratinocytes by >90% beginning at times as short as 30 min after drug treatment.

[000117] Table 5 presents a summary of measured IC_{50} values and IC_{90} values of certain polyamides against HPV16, HPV18 and HPV31. The polyamides were tested in cells that maintain HPV16, HPV18 or HPV31 DNA. Cells maintaining the selected HPV were cultured for 72 hours in the presence of the polyamide. Viral DNA was then quantified using real-time PCR and compared to vehicle (DMSO)-treated control cultures. The results obtained demonstrate that the tested polyamides generally exhibited effectiveness in inhibiting replication of HPV16, HPV18 and HPV31.

Table 5. Activity of polyamides against HPV

NanoVir #	HPV16 IC50 [μM]	HPV16 IC90 [μM]	HPV18 IC50 [μM]	HPV18 IC90 [μM]	HPV31 IC50 [μM]	HPV31 IC90 [μM]
NV1002	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1002*	0.554 (± 0.002)	2.347	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED
NV1002*	0.343 (± 0.027)	3.076	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED
NV1003	0.235	0.813	0.723	6.821	0.340	1.125
NV1004	0.152	0.674	0.070	0.997	0.556	0.929

NV1005	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1006	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1007***	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1008***	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1009***	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED
NV1010	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1011	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1012	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1013	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1014	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1015	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1016	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1017	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1018	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1019	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1020	4.984	>10	NOT TESTED	NOT TESTED	0.301	2.626
NV1021	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1022	5.206	9.332	NOT TESTED	NOT TESTED	2.195	>10
NV1023	5.002	8.991	NOT TESTED	NOT TESTED	0.858	3.946
NV1024	4.933	9.374	7.058	>10	0.888	6.435
NV1025	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT ACTIVE	NOT ACTIVE
NV1026	1.011	>10	NOT ACTIVE	NOT ACTIVE	0.705	6.878
NV1027	0.219	0.640	0.398	2.12	0.165	0.777
NV1028	0.100	1.113	0.717	>10	0.108	0.986
NV1029	0.133	0.917	2.571	>10	0.261	8.950
NV1030	0.131	1.326	0.415	>10	0.127	1.865
NV1031	0.378	2.012	0.470	9.191	0.244	0.983

NV1032	0.315	4.057	NOT ACTIVE	NOT ACTIVE	0.165	2.169
NV1033	2.035	>10	NOT ACTIVE	NOT ACTIVE	2.990	>10
NV1037	0.146	0.773	0.092	0.871	0.027	0.200
NV1038	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1039	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1040	0.994	6.730	0.579	>10	0.340	6.615
NV1041	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1042	0.036	0.351	0.056	1.462	0.030	0.510
NV1043	7.039	>10	0.456	10	5.223	>10
NV1044	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1045	1.378	>10	0.300	>10	1.648	8.926
NV1046	0.250	3.050	0.190	>10	0.119	5.278
NV1047	1.549	8.561	4.277	>10	3.943	8.91
NV1048	0.137	4.226	0.411	>10	0.061	0.882
NV1049	0.659	7.145	0.258	3.432	1.242	7.348
NV1050	0.600	>10	0.997	>10	0.820	>10
NV1051**	3.97	>10	NOT ACTIVE	NOT ACTIVE	0.879	>10
NV1052**	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1053**	2.240	>10	NOT ACTIVE	NOT ACTIVE	0.468	1.845
NV1054	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1055	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1056	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1057#	NOT ACTIVE	NOT ACTIVE	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED
NV1058	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1059	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1060	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1061	0.878	5.629	2.143	>10	1.152	9.518
NV1062	0.199	0.400	NOT ACTIVE	NOT ACTIVE	0.273	0.608
NV1063	0.137	0.334	NOT ACTIVE	NOT ACTIVE	0.100	0.189
NV1064	1.295	4.062	NOT ACTIVE	NOT ACTIVE	0.688	1.338

NV1065	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1066	0.132	0.835	0.280	1.589	0.141	1.747
NV1067	0.409	1.210	0.389	2.092	0.205	2.119
NV1068	0.220	2.311	0.516	5.205	0.314	4.067
NV1069	0.725	2.257	1.509	8.744	0.363	0.894
NV1070	0.308	4.898	0.459	10	0.398	2.157
NV1071	0.255	2.317	0.093	3.809	0.095	1.597
NV1072	0.109	>10	0.216	>10	0.096	10
NV1073	0.267	10	0.405	>10	0.220	>10
NV1074	0.178	1.749	0.041	0.192	0.052	0.320
NV1075	0.124	2.627	0.049	0.314	0.056	0.546
NV1076	0.067	0.594	0.048	0.236	0.104	0.403
NV1077	0.095	0.410	0.015	0.094	0.032	0.181
NV1078	0.046	0.307	0.030	0.091	0.047	0.176
NV1079	0.041	0.152	0.023	0.115	0.041	0.150
NV1080	0.058	0.288	0.037	0.113	0.073	0.168
NV1081	NA	NA	NA	NA	NA	NA
NV1082	NA	NA	NA	NA	NA	NA
NV1083	2.41	>10	0.929	>10	3.082	>10
NV1084	1.91	>10	1.041	>10	7.325	>10
NV1085	0.029	0.353	0.041	0.201	0.032	0.213
NV1086	0.043	0.212	0.062	0.174	0.016	0.174
NV1087	0.031	0.200	0.024	0.297	0.016	0.135
NV1088	0.02	0.180	0.035	0.157	0.014	0.136
NV1089	0.068	0.265	0.068	0.169	0.046	0.217
NV1090	0.051	0.215	0.053	0.617	0.022	0.157
NV1092	0.169	0.962	2.9	>10	0.03	0.088
NV1093	0.072	0.970	0.085	2.6	0.042	0.335
NV1094	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1095	0.204	2.196	NOT ACTIVE	NOT ACTIVE	0.049	0.324
NV1096	0.024	0.138	0.036	0.348	0.035	0.090
NV1097	0.07	1.407	0.257	>10	0.04	10
NV1098	0.011	1.360	0.017	>10	0.024	0.549
NV1101	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED
NV1102	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1103	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1104	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1105	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE

NV1106	6.7	NOT TESTED	NOT ACTIVE	NOT ACTIVE	1.4	NOT TESTED
NV1107	0.028	0.180	0.025	0.340	0.018	0.276
NV1108	0.212	0.718	0.513	>10	0.673	6.752
NV1109	0.055	0.293	0.075	0.290	0.045	0.267
NV1110	0.43	4.445	0.921	>10	0.825	>10
NV1111	0.017	0.121	0.018	0.136	0.004	0.079
NV1112	0.047	0.195	0.057	0.324	0.022	0.151
NV1113	0.304	>10	0.228	>10	0.125	0.888
NV1114	0.035	0.411	0.036	0.125	0.009	0.085
NV1115	0.103	0.378	0.167	0.937	0.139	0.752
NV1116	0.038	0.340	0.029	0.142	0.016	0.127
NV1117	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE	NOT ACTIVE
NV1118	0.018	0.245	0.007	0.254	0.017	0.162
NV1119	0.058 ± 0.009	0.553 ± 0.154	0.027 ± 0.0001	0.191 ± 0.002	0.035 ± 0.00002	0.106 ± 0.0005
CHC2001	0.026 ± 0.004	0.299 ± 0.082	0.106 ± 0.002	1.489 ± 1.234	0.072 ± 0.003	0.384 ± 0.019
CHC2002	0.636 ± 0.606	2.305 ± 7.486	0.758	>10	0.058 ± 0.009	0.341 ± 0.470
KJK6045f 26-28	0.817 ± 0.535	2.279 ± 6.981	8.726	>10	1.775 ± 9.062	>10
KJK6047-1	1.471 ± 15.186	>10	6.011 ± 4.571	>10	1.046 ± 6.472	>10
KJK6048	2.048	>10	9.172	>10	1.859 ± 6.809	7.344 ± 15.156
KJK6049	0.435 ± 0.073	2.249 ± 7.107	>10	>10	0.258 ± 0.040	1.253 ± 0.189
KJK6050f 16-18	0.400 ± 0.426	3.035 ± 12.939	0.259 ± 0.113	5.916	0.664 ± 2.507	1.688 ± 18.426
KJK6062	0.227 ± 0.036	2.966 ± 9.136	0.155 ± 0.001	>10	0.518 ± 0.352	1.876 ± 5.317
KJK6065f 17-19	0.066 ± 0.009	0.472 ± 0.136	2.456 ± 4.818	>10	0.571 ± 0.111	2.233 ± 3.395
KJK6067f 12	0.191 ± 0.006	2.331 ± 2.506	0.979 ± 1.496	4.007 ± 5.165	0.445 ± 0.171	1.518 ± 11.392
KJK6068f 20-22	1.556	>10	1.151 ± 1.887	>10	0.407 ± 0.0002	1.151 ± 0.011
KJK6076	0.047 ± 0.001	0.187 ± 0.005	0.041 ± 0.0003	0.147 ± 0.019	0.019 ± 0.0001	0.090 ± 0.0003
KJK6099	0.289 ± 0.058	>10	6.462	>10	0.403 ± 0.031	2.145 ± 0.193
NV1042	0.036	0.351	0.056	1.462	0.030	0.510

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[000118] In Table 5, "NA" or "NOT ACTIVE" indicates no measurable antiviral response was obtained relative to control at the highest dose tested (10 µM). The IC_{50} is the concentration of compound required for 50% decrease of viral DNA concentration *in vitro*. The IC_{90} is the concentration of compound required for 90% decrease in viral DNA concentration *in vitro*.

[000119] The results demonstrate that the polyamides of the invention exhibited effectiveness in inhibiting replication of HPV16, HPV18 and HPV31. Thus, the polyamides of the invention are demonstrated to exhibit activity against HPV in human cells which are infected with HPV and may represent an effective therapeutic agent for the treatment of patients infected with HPV or other papillomaviruses.

[000120] Polyamides designated in Table 5 as KJK6045f26-28; KJK6047-1; KJK6050f16-18; KJK6062; KJK6065f17-19; and KJK6067f12 are TMG or unsubstituted guanidine (Guan) derivatives.

[000121] The data show that the compounds with high anti-HPV IC_{90} values are TMG and unsubstituted guanidine (Guan) derivatives. An IC_{90} value is the single most important metric used in the antiviral drug discovery arena, and the fact that TMG and unsubstituted guanidine (Guan) derivatives exhibit high IC_{90} values is surprising and is in no way predictable from the prior art.

[000122] What is more, the results show that N-terminal substitution of an anti-HPV polyamide with unsubstituted Guan or substituted Guan derivatives improves the antiviral activities against the two most prevalent high-risk HPV types (HPV16 and -18). Upon N-terminal substitution with a tetramethylguanidinium group, improvement in anti-HPV activity (IC_{50}) was observed against HPV18.

[000123] Polyamides designated in Table 5 as CHC2001; CHC2002; KJK6045f26-28; KJK6047-1; KJK6048; KJK6049; KJK6050f16-18; KJK6062; KJK6065f17-19; KJK6067f12; KJK6068f20-22; KJK6076; FT1138; and FT1139, are formate salts of polyamide compounds.

[000124] Moreover, the compounds of the invention include formate salts of polyamide compounds. Table 5 shows the anti-viral activity of the polyamide compound NV1028 as well as the formate salts of NV1028 which is designated as CHC2001. Moreover, Table 5 shows the anti-viral activity of NV1042 as well at the formate salts of NV1042 designated as KJK6076.

[000125] Surprisingly, the polyamide compounds in the form of a formate salt exhibit enhanced activity over those compounds which are not in the formate salt form. For example, a comparison of the IC_{90} value of NV1028 and CHC2001 demonstrates a nearly 4-fold increase in the IC_{90} value when the polyamide is in the formate salt form. Such enhanced antiviral activity observed with the formate salt form of the polyamide is unexpected and may not be predicted based on the use of formate salts in chemical synthesis *per se*.

[000126] What is more, is was observed that NV1106 exhibits anti-viral activity, which anti-viral activity is remarkable given that NV1106 is only an 8-ring polyamide.

[000127] Several alternative approaches may be used to confirm the effects of the compounds on viral DNA. These additional procedures include normalization to total DNA, preparation of DNA by different procedures including DNeasy (Total Genomic DNA) Qiagen spin columns, DNAzol total genomic DNA preparations, and Hirt (low MW DNA preparations; (Hirt, (1967), J Mol Biol. 26:365-9).

[000128] Southern blotting may be used to confirm the effects of polyamides on HPV DNA levels that were determined using real-time PCR technology. The experiments

may be conducted as previously described (Gamer-Hamrick and Fisher, Virology, 301, 334-41, 2002).

[000129] The toxicity of each polyamide found active against HPV may be monitored in normal human keratinocytes using an MTT cell viability assay (Denizot and Lang, 1986).

[000130] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

EXAMPLES

[000131] EXAMPLE 1: A single polyamide dose impedes infection by polyomavirus and promotes cell survival.

[000132] A representative polyamide, NV1042, was prepared. BS-C-1 cells were plated at 5 x 10^5 cells per well in 24-well plates in culture media composed of 10% FBS plus 50 U/mL penicillin-streptomycin (Life Technologies, cat # 15070063). Upon reaching 90-100% confluency, SV40 virus was diluted in MEM-2% FBS and 50 µL was applied per well at a multiplicity of infection (MOI) equal to 1. Virus was allowed to adhere to cells for 2h at 37ºC in a humidified incubator with 5% CO_2 at which time virus was removed and 0.5 mL MEM-2% FBS was added to each well. Infection was allowed to proceed for 24 h before 10 µM NV1042 in 0.1% DMSO was added to culture wells and incubated an additional 72 h before being removed by a change of media. The effects of polyamide treatment was followed for up to 12 days post-infection. The cells were examined each day for effects on cell survival and substratum adherence (a measure of viability). Cells that were not infected by SV40 showed no signs of cell death or loss, while SV40 infected cells began dying due to viral cytopathic effect (CPE) by 6-7 days post infection. By 9 days post-infection the virus-induced death of cells was

complete and no adherent cells remained. On the other hand, the single polyamide treatment resulted in significant protection of cells from CPE so that even by 12 days post-infection cell survival was clearly noticeable.

[000133] A study of SV40 virus DNA levels over the 12 day period of infection clearly demonstrated that the single polyamide treatment potently blocked viral DNA propagation. Following the initial infection, DNA was extracted each day from all adherent and floating cells with DNAzol (Life Technologies, cat # 10503-027) according to the manufacturer's recommendation. Q-PCR reaction mixtures contained 8 µL sample DNA, 200 nM each primer, 250nM probe and 10 µL 2X Master Mix (LightCycler 480 Probes Master; Roche, cat # 04887301001) in a total volume of 20 µL. Samples were analyzed using instrumentation purchased from Roche (LightCycler 480). Drug effects on viral DNA copy number were calculated as a percentage of viral DNA copies quantified in the DMSO-treated cells (standard-curve method), the log-dose response plotted, and IC_{50}s calculated using best-curve fitting software (XLfit; IDBS, United Kingdom). The primers and probe for SV40 were as follows: forward 5'-CCTCCGTTAAGGTTCGTAGGTCA-3' (SEQ ID NO:1), reverse 5'-CAGCGCTCACACCAGTC-3' (SEQ ID NO:2) probe 5'-/56-FAM-ATTGAAGTGCAACTTGCATCTGTTGCTACTGTTGA-3BHQ_1/-3' (SEQ ID NO:3). Cycling conditions: 10 min at 95ºC followed by 40 cycles of 95ºC for 30 sec and 60ºC for 60 sec. A single polyamide treatment caused a profound inhibition of viral DNA levels for the period of infection relative to the untreated cells so that by day 12 (11 days after polyamide treatment) only 25% of the viral DNA levels found in the untreated samples was present in the treated samples.

[000134] The delay in virus propagation was clearly evident and demonstrable by following the expression of large T antigen (LT, the primary transforming protein of SV40 involved in virus replication) and phosphorylation of ATM, the DNA repair protein activated by SV40 and large T antigen. Cells were fixed in 3.7% paraformaldehyde for 5 min at RT and washed three times with PBS. Cells were then permeabilized in 0.2% Triton X-100 in PBS for 5 min at RT and washed 3X with PBS. Prior to IF, nonspecific

antigen binding was blocked by incubation of cells with 3% BSA in PBS for 30 min at RT. Primary antibody dilutions were prepared in 1% BSA-PBS; LT-ag was localized with PAb416 (Abcam, cat # ab16879) at 1:200 dilution and Phospho S1981 ATM (pATM) with EP1890Y (Abcam, cat # ab81292) at 1:250 dilution with RT incubation for 2h. Coverslips were washed 3X for 10 min each with PBS. Secondary antibodies were diluted in 1% BSA-PBS at 1:500 dilution; LgT-ag was detected with goat anti-mouse AlexaFluor 546 (Life Technologies, cat # A11056) and pATM with goat anti-rabbit AlexaFluor 488 (Life Technologies, cat # A11055). Coverslips were incubated with secondary antibodies for 2h at RT and then washed 3X 10 min each with PBS. Total nuclei were visualized by staining with DAPI contained in the mounting solution (ProLong Gold with DAPI; Life Technologies, cat # P36931). Expression of LT and pATM were both significantly delayed following the single polyamide treatment in a manner that reflected both the delay in cell death and viral DNA propagation. The results were quantifiable and correlated well with cell loss from CPE and cell protection by the polyamide.

[000135] EXAMPLE 2: Effects of representative polyamides on various DNA viruses

[000136] The effects of polyamides were tested on a variety of DNA viruses that were either maintained in cells as episomes (HPV16 in W12 cells or EBV in Raji cells) or were introduced into cells following infection (BKV and SV40). For SV40: BS-C-1 cells were plated at 5 x 10^5 cells per well in 24-well plates. Upon reaching 90-100% confluency, SV40 virus was diluted in MEM-2% FBS and 50 µL was applied per well at a MOI equal to 1. Virus was allowed to adhere to cells for 2h at 37 ºC in a humidified incubator with 5% CO_2 at which time virus was removed and 0.5 mL MEM-2% FBS was added to each well. Infection was allowed to proceed for 24 h before increasing doses of PA from 0.001-10 µM or 0.1% DMSO was added to culture wells and incubated an additional 48 h. Following the treatment period, DNA was extracted with DNAzol (Life Technologies, cat # 10503-027) according to the manufacturer's recommendation. For BKV: RPTEC cells were plated at 1 x 10^5 cells per well in 24-well plates in renal epithelial cell growth medium (REGM; ATCC-PCS-400-030) supplemented with

recombinant human epidermal growth factor (10 ng/mL), triiodothyronine (10 nM), hydrocortisone (100ng/mL), recombinant human insulin (5 µg/mL), epinephrine (1 µM), transferrin (5 µg/mL), 0.5% fetal bovine serum (renal epithelial cell growth kit; ATCC-PCS-400-040) and GA-1000 (30 µg/mL gentamicin and 15 ng/mL amphotericin B; Lonza cat # CC4083). When confluent, cells were infected with 50 µl BKV-TU or BKV-DUN per well at an MOI of 0.5 FFU/mL diluted in REGM with 0.5% FBS. Following 2 h incubation, virus inoculum was removed and replaced with 500 µL of fresh REGM-0.5% FBS. BKV infected RPTEC cells were cultured for 48 h and then increasing doses of PA from 0.001-10 µM or 0.1% DMSO was added to culture wells and incubated an additional 48 h, at which time DNA was extracted with DNAzol. For EBV: Raji cells were cultured in 12-well plates at a density of 2 x 10^5 cells/mL in RPMI-10% FBS containing either increasing doses of PA from 0.001-10 µM or a vehicle control of 0.1% DMSO. Following a 48 h treatment period, cells were centrifuged at 1000 rpm for 3 min and DNA extracted with DNAzol. DNA concentration and purity was determined spectrophotometrically and diluted to 20 ng in 8 µL dH_2O for Q-PCR.

[000137] HPV16 DNA episomes were studied in W12 cells as previous described(33).

[000138] The smaller viruses (HPV, SV40, and BKV) all responded to the test polyamide NV1042 with IC_{50}'s in the low nanomolar range, indicating that these viruses are good therapeutic targets for polyamide NV1042 and related polyamides and polyamide classes described herein.

[000139] Moreover, the low nanomolar IC_{50} range indicates that the compounds may be administered in a therapeutically effective amount for efficacious treatment of human papillomavirus and polyomavirus infected subjects/patients.

[000140] EXAMPLE 3: IC_{50}'s and IC_{90}'s of polyamides tested against SV40 and BKV.

[000141] A series of polyamides was tested in antiviral assays against polyomaviruses as described above. Table 4 ranks the polyamides by potency (IC_{50}) against the

prototypical polyomavirus SV40. The two polyamides not tested against SV40, NV1089 and NV1090, were highly potent against representative BKV strains. In general, if a polyamide demonstrated good activity against SV40 it also exhibited potent activity against BKV. Conversely, those polyamides with poor activity against SV40 (>0.5μM IC_{50}) also showed poor or no activity against BKV. For polyamides tested and listed in Table 4 and Table 5, the sequences are shown in Table 1 and their high resolution mass spectral data are given in Table 2 and Table 3 for verifying composition.

[000142] From these results, one skilled in the art may conclude that the polyamides of the invention would provide therapeutic efficacy in clinically relevant polyomaviruses.

[000143] EXAMPLE 4: Polyamides may work through a mechanism that involves the DNA damage response (DDR) and inhibition of members of DDR pathways that regulate DNA strand break repair act as polyamide enhancers.

[000144] BS-C-1 and RPTEC cells were plated and infected as above with SV40 and BKV respectively. After 24 h increasing doses of NV1042 from 0.001-10 μM was added in the presence or absence of 100 μM Mirin (Sigma, cat # M9948; an inhibitor of MRE11) or 0.1% DMSO was added for an additional 48 h. At the end of the treatment period, total DNA was harvested with DNAzol and 20 ng total DNA analyzed by Q-PCR. These studies demonstrate that the IC_{50} of polyamide against 2 different polyomaviruses, SV40 and BKV, is enhanced (i.e. lowered) by inhibition of Mre11, which is important for ssDNA and dsDNA break repair(34).

[000145] Inhibitors of Chk2 also acted as enhancers of polyamide activity. BS-C-1 cells were seeded at 4 x 10^5 cells per 60mm dish and cultured for 72 h until 90-100% confluent. Cells were infected with SV40 virus in MEM + 2% FBS for 2 h at 37 ºC (200 μL virus/p60 at 100 pfu/mL; MOI=0.00001), at which time virus-containing media was removed and cells incubated with fresh media for 24 h. Virus-infected cells were then treated with 0.1% DMSO, 1 μM NV1042, 10 μM NV1042, 5 μM Chk2-inhibitor (Chk2-I; Sigma, cat # C3742). Media was changed after 4 days of drug treatment and cultures

maintained for an additional 10 days. Plates were then stained for 10' with 0.1% Crystal Violet (Ted Pella, cat # 18711) plus 1% formaldehyde, 1% Methanol, and PBS, rinsed with tap water until water ran clear and air-dried and photographed. Chk2-I acted to enhance the antiviral activity of 1 μM NV1042 as evidenced by the protection of BS-C-1 cells from SV40 CPE relative to 1 μM NV1042 alone.

[000146] EXAMPLE 5: Effects of representative polyamides on HPV.

[000147] A series of polyamides are tested in antiviral assays against human papillomavirus. The antiviral activities are calculated by measuring the ability of the polyamide compounds to decrease the HPV viral episomal load in monolayer keratinocyte cultures. The episomal load is determined by Q-PCR.

[000148] The potency (IC_{50} and IC_{90}) of the polyamides of the invention against HPV is evaluated as described above. As shown in Table 5, the polyamides of the invention exhibit activity against HPV in human cells which are infected with HPV. Thus, the polyamides of the invention may represent an effective therapeutic agent for the treatment of patients infected with HPV.

* * * * *

[000149] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description.

[000150] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference.

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CLAIMS

1. A compound of the formula:

Guan-PPPβPPβPIm–γ–PβPPβPPPβPβTa;

TMG-PPβPPβPIm–γ_{NH2}–PβPPβPPPβPPTa;

Guan-PPPβPPβPIm–γ–PβPPβPPPβPβDp;

TMG-PβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβPIm–γ_{NHR}–PβPPβPPPβPβTa;

ImPPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβPIm–γ–PβPPβPPPβPβDp;

TMG-PPPβPPβPIm–γ–PβPPβPPPβPβTa;

Guan-PPβPPImβPP–γ–PPβPPPβPPPβTa;

TMG-PPPβPPβP–γ–PPPβPPPβPβ-Ta;

ImPPβPPImβPP–γ–PPβPPPβPPPPTa;

TMG-PPPβPPβPIm–γ_{NH2}–PβPPβPPPβPβTa;

TMG-PPβPPβP–γ–PPPβPPPβPβTa;

Guan-IPPβPPIβPP–γ–PPβPPPβPPPβTa;

TMG-IPPβPPIβPP–γ–PPβPPPβPPPβTa;

ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβDp;

TMG-PPβPPImβPP–γ–PPβPPPβPPPβDp;

TMG-PPPβPPbP–$\gamma_{NHR'}$–PPPβPPPβPβDp;

ImPPβPPImβP–γ–PPβPPPβPPPβTa;

ImPPβPPImβPP–γ_{NH2}–PPβPPPβPPPβTa;

TMG-PβPPImβPP–γ–PPβPPPβPPPβDp;

Guan-PPβPPImβPP–γ–PPβPPPβPPPβDp;

Guan-IPPβPPP–γ–PPβPPPPβTa;

ImPPβPPP–γ–PPβPPPPβTa;

TMG-IPPβPPP–γ–PPβPPPPβTa;

ImPPβPP–γ–PPβPPPPβTa;

ImPPβPPP–γ_{NH2}–PPβPPPPβTa;

ImPPβPPImβPP–γ_{NHAc}–PPβPPPβPPPβDp;

Guan-PPβPPP–γ–PPβPPPPβTa;

ImPPPβPPβ–γ–PPβPPPβPPDp;

TMG-PPβPPP–γ–PPβPPPPβTa; or

Guan-PPβPPP–γ–PPβPPPPβDp,

wherein GUAN = a guanidinyl radical; TMG = tetramethylguanidinyl;
P = 4-amino-2-carbonyl-N-methylpyrrole; γ = gamma-aminobutyric acid;
γ_{NH2} = (R)-2,4-diaminobutyric acid reacted through either the 2-amino group or the 4-amino group; γ_{NHAc} = (R)-2-(acetylamino)-4-aminobutyric acid;
β = beta-alanine; Im = 4-amino-2-carbonyl-N-methylimidazole;
Ta = 3,3'-diamino-N-methyldipropylamine; and Dp = (dimethylamino)propylamine.

2. The compound of Claim 1 which is in the form of a formate salt.

3. The use of a compound of Claim 1 in a medicament for the treatment of polyomavirus infected cells.

4. A method of treating cells infected with a polyomavirus comprising administering to a subject infected with the polyomavirus, a therapeutically effective amount of a compound of Claim 1.

5. A compound of the formula:

ImPPPβPP-γ-PPPβPPPβDp;

ImPPPβPPβPPP-γ_{NH2}-PPPβPPPβPPPβDp;

ImPPPβPPβPPP-γ-PPPβPPPβPPPβDp;

ImPPPβPPP-γ_{NH2}-PPPβPPPββDp;

ImPPPβPPβ-γ-PPβPPPβPβTa;

ImPPβPPP-γ-PPβPPPPβTa;

ImPPPβPPβ-γ_{NH2}-PPβPPPβPβDp;

ImPPβPPP-γ_{NH2}-PPβPPPPβTa;

ImPPPβPPP-γ-PPPβPPPββTa;

ImPPPβPPβ-γ_{NH2}-PPβPPPβPβTa;

ImPPβPImPβPP-γ_{NH2}-PPβPPPβPPPβTa;

ImPPβPPImβPP-γ-PPβPPPβPPPβDp;

ImPPβPPImβPP-γ-PPβPPPβPPPβTa;

ImPPPIm-γ-βPPPPβDp;

ImPPPIm-γ-βPPPPβTa;

ImPPβPImPβPPIm-γ-βPβPPPβPPPβTa;

ImPβPPβPPImβPPP-γ-PβPPβPPPβPPPPβTa;

ImPPPβPPPβPP-γ-PPPβPPPβPPPβDp;

ImPPPβPPPβPP-γ-PPPβPPPβPPPβTa;

ImPPβPPImβPPαPPβPPPβPPPβTa (5 TFA);

ImPPβPPImβPPαNH2PPβPPPβPPPβDp (4 TFA);

ImPPβPPImβPPαNHAcPPβPPPβPPPβTa (4 TFA);

ImPPPImαNH2βPPPPβTa (5 TFA);

ImPPβPPPαNHAcPPβPPPPβTa (3 TFA);

ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβDp;

ImPPβPPImβPP-γ_{NH2}-PPβPPPβPPPβTa;

ImPPβPPImβPP-γ_{NHAc}-PPβPPPβPPPβDp;

ImPPβPPP-γ-PPβPPPPPTa • 3TFA;

ImPPβPPImβPP-γ-PPβPPPβPPPPTa • 4TFA;

TMG -PPβPPβPIm-γ_{NH2}-PβPPβPPPβPPTa • 5TFA;

TMG -PPPβPPβPPβPIm-γ_{NHR}-PβPPβPPPβPPβPβTa • 6TFA;

TMG -PPPβPPβPPβPIm-γ_{NH2}-PβPPβPPPβPPβPβTa • 5TFA;

TMG -PPPβPPβPP-γ_{NHR}-PPPβPPPβPPβPβTa • 5TFA;

TMG -PPPβPPβPPβPIm-γ_{NH2}-PβPPβPPPβPPβPβDp • 4TFA;

TMG -PPPβPPβPP-γ_{NH2}-PPPβPPPβPPβPβTa;

TMG -PPPβPPβPP-γ_{NH2}-PPPβPPPβPPβPβDp;

TMG –PβPPImβPP-γ-PPβPPPβPPPβPβTa • 4TFA;

TMG -PPPβPPβPP-$\gamma_{NHR'}$-PPPβPPPβPPβPβDp • 3TFA;

TMG –PβPPImβPP-γ-PPβPPPβPPPβPβDp • 3TFA;

TMG –PPβPPP-γ-PPβPPPPPβDp • 2TFA;

TMG –PPβPPP-γ-PPβPPPPPβTa • 3TFA;

TMG –PPβPPImβPP-γ-PPβPPPβPPPβPβTa;

TMG –PPβPPImβPP-γ-PPβPPPβPPPβPβDp;

TMG –PPPβPPβPPIm-γ-PβPPβPPPβPPβPβTa;

TMG –PPPβPPβPPIm-γ-PβPPβPPPβPPβPβDp;

TMG –PpβPPβPPIm-γ-PβPPβPPPβPPβPβDp;

TMG –PpβPPβPPIm-γ-PβPPβPPPβPPβPβTa;

ImPPβPP-γ-PPβPPPPPβTa;

ImPPβPPImβP-γ-PPβPPPβPPPβPβTa;

TMG –PPβPPβP-γ_{NH2}-PPPβPPPβPPβPβTa;

TMG –PpβPPβPPIm-γ_{NH2}-PβPPβPPPβPPβPβTa;

TMG –PPβPPβP-γ-PPPβPPPβPPβ-Ta • 3TFA;

TMG –PPPβPPβP-γ-PPPβPPPβPPβ-Ta • 3TFA;

Guan-PPβPPImβPP-γ-PPβPPPβPPPβPβTa (4 TFA);

Guan-PPβPPP-γ-PPβPPPPPβTa (3 TFA);

Guan-PPβPPImβPP-γ-PPβPPPβPPPβPβDp (3 TFA);

Guan-PPβPPP-γ-PPβPPPPPβDp (2 TFA);

Guan-PPPβPPβPPImγPβPPβPPPβPPβPβTa (4 TFA);

Guan-PPPβPPβPPIm-γ-PβPPβPPPβPPβPβDp (3 TFA);

TMG –IPPβPPP-γ-PPβPPPPPβTa (4 TFA);

TMG –IPPβPPIβPP-γ-PPβPPPβPPPβPβTa (5 TFA);

Guan-IPPβPPP-γ-PPβPPPPβTa (4 TFA);

Guan-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA);

Ac-IPPβPPIβPP-γ-PPβPPPβPPPβTa (5 TFA);

PPβPPβPIm-γ-PβPPβPPPβPβTa;

ImPPβPPP-γ-PPβPPPPβTa (3 HCO2H);

ImPPPβPP-γ-PPPβPPPβTa (3 HCO2H);

TMG –ImPβPPP-γ-PβPPPPβTa (4 HCO2H);

Guan-ImPβPPP-γ-PβPPPPβTa (4 HCO2H);

ImPβPPP-γ-PβPPPPβTa (3 HCO2H);

ImPPβPP-γ-PPβPPPβTa (3 HCO2H);

TMG –ImPPβPP-γ-PPβPPPβTa (4 HCO2H);

Guan-ImPPβPP-γ-PPβPPPβTa (4 HCO2H);

TMG –PPImβPP-γ-PPβPPPβTa (4 HCO2H);

Guan-PPImβPP-γ-PPβPPPβTa (4 HCO2H);

PPImβPP-γ-PPβPPPβTa (3 HCO2H);

ImPPβPPImβPP-γ-PPβPPPβPPPβTa (4 HCO2H);

ImPPPβPP-γ-PPPβPPPβDp (2 TFA);

ImPPβPPImβPP-γ-PPβPPPβPPPβTa-AF488 (HCO_2H); or

ImImPIm-γ-PβPPβTa-AF488 (2 HCO_2H),

wherein GUAN = a guanidinyl radical;TMG = tetramethylguanidinyl;
P = 4-amino-2-carbonyl-N-methylpyrrole; γ = gamma-aminobutyric acid;
γ_{NH2} = (R)-2,4-diaminobutyric acid reacted through either the 2-amino group or the 4-amino group; γ_{NHAc} = (R)-2-(acetylamino)-4-aminobutyric acid; β = beta-alanine; Im = 4-amino-2-carbonyl-N-methylimidazole;Ta = 3,3'-diamino-N-methyldipropylamine; Dp = (dimethylamino)propylamine; TFA = trifluoroacetic acid; HCO_2H = formate; AF488=ALEXA-488 fluorophore; and α means that the γ-aminobutyric acid formed an α-linked hairpin rather than the typical γ-linked hairpin; and AF488= AlexaFluor-488 fluorophore.

6. The compound of Claim 5 which is in the form of a formate salt.

7. The use of a compound of Claim 5 in a medicament for the treatment of papillomavirus infected cells.

8. A method of treating cells infected with a papillomavirus comprising administering to a subject infected with the papillomavirus, a therapeutically effective amount of a compound of Claim 5.

Figure 1

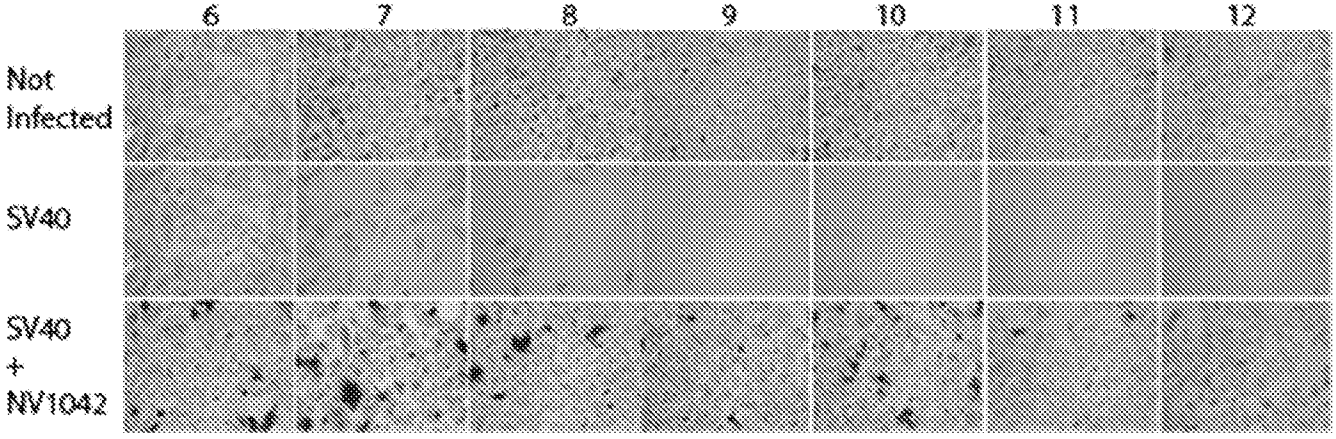


Figure 2

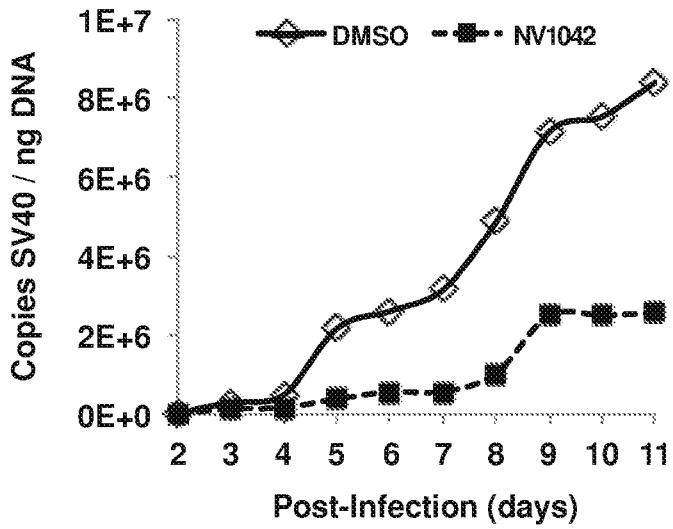

1E+7
8E+6
6E+6
4E+6
2E+6
0E+0
Copies SV40 / ng DNA
DMSO
NV1042
2 3 4 5 6 7 8 9 10 11
Post-Infection (days)

Figure 3

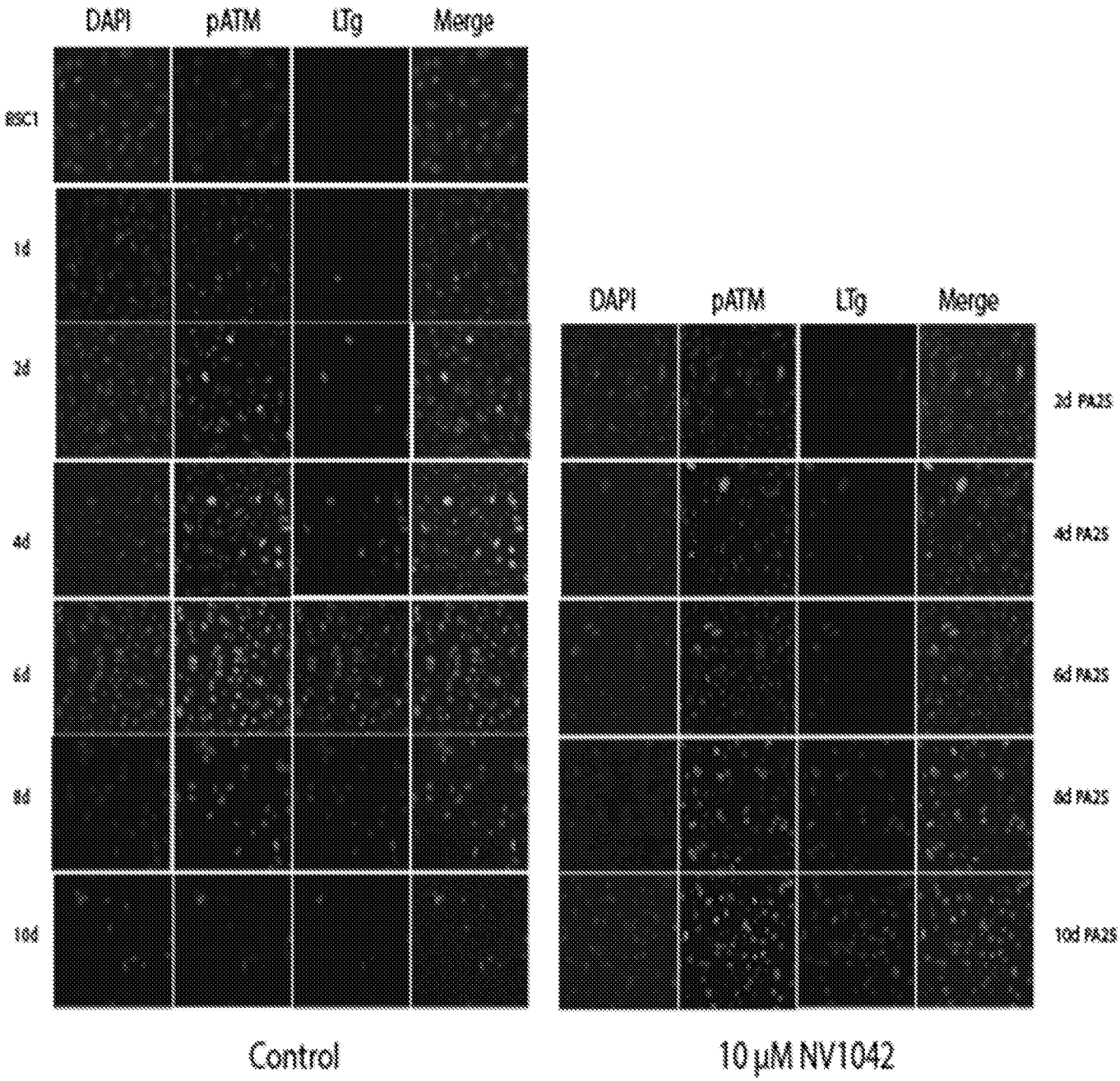

DAPI
pATM
LTg
Merge
DAPI
pATM
LTg
Merge
3d PA25
4d PA25
6d PA25
8d PA25
10d PA25
Control
10 μM NV1042

Figure 4

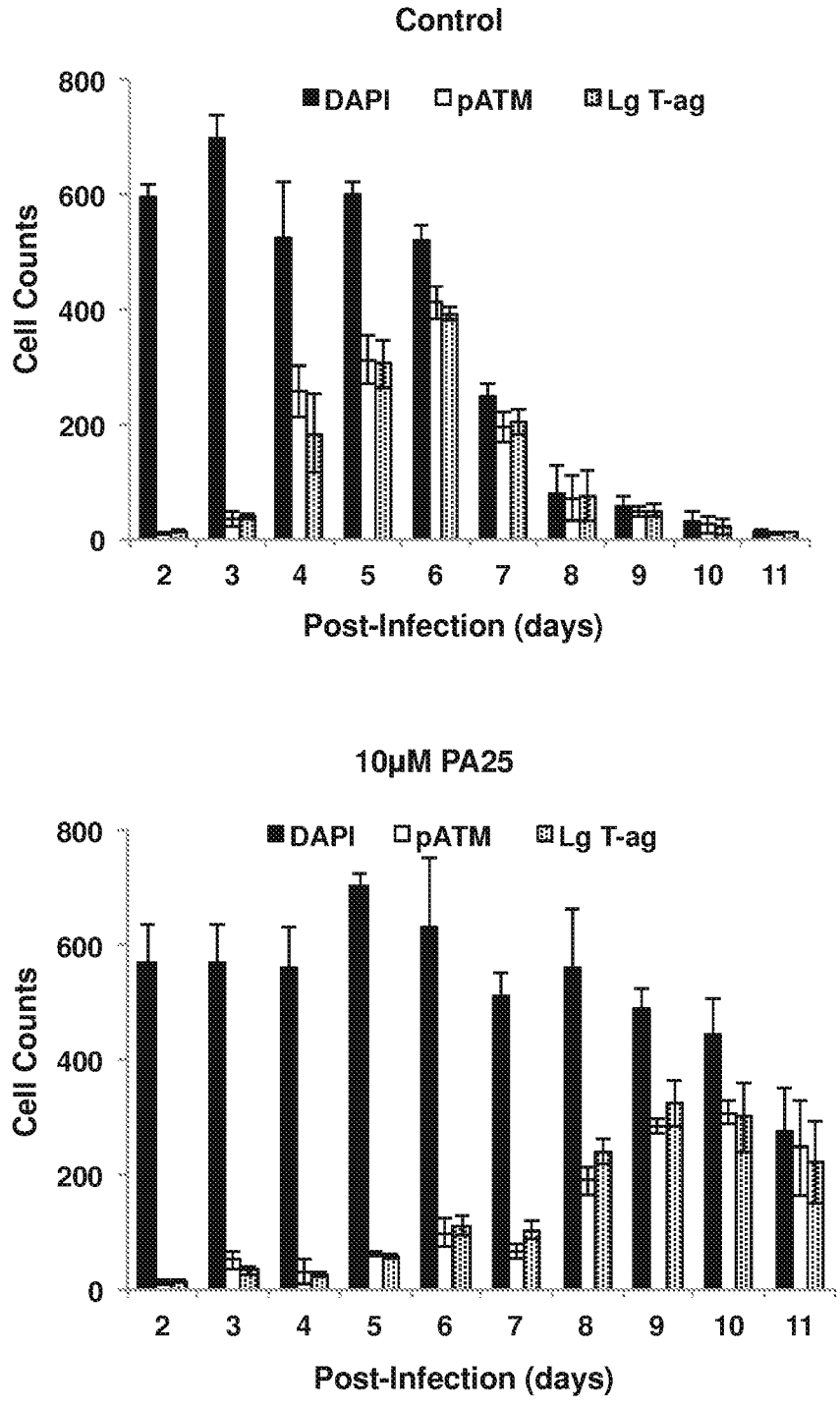

Control
DAPI
pATM
Lg T-ag
Cell Counts
800
600
400
200
0
2
3
4
5
6
7
8
9
10
11
Post-Infection (days)
10μM PA25
DAPI
pATM
Lg T-ag
Cell Counts
800
600
400
200
0
2
3
4
5
6
7
8
9
10
11
Post-Infection (days)

Figure 5

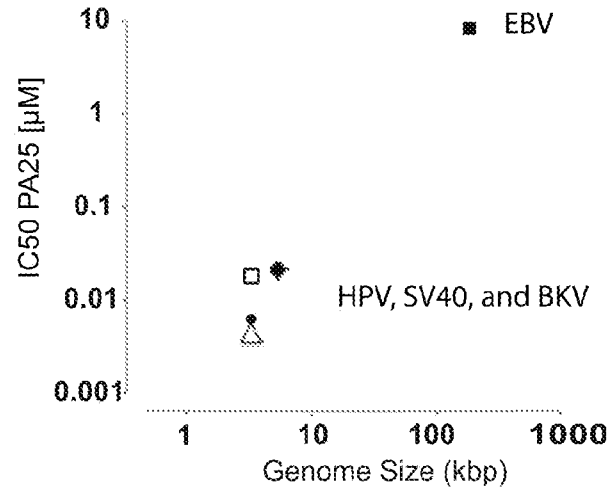

10
1
0.1
0.01
0.001
IC50 PA25 [µM]
EBV
HPV, SV40, and BKV
1
10
100
1000
Genome Size (kbp)

Figure 6

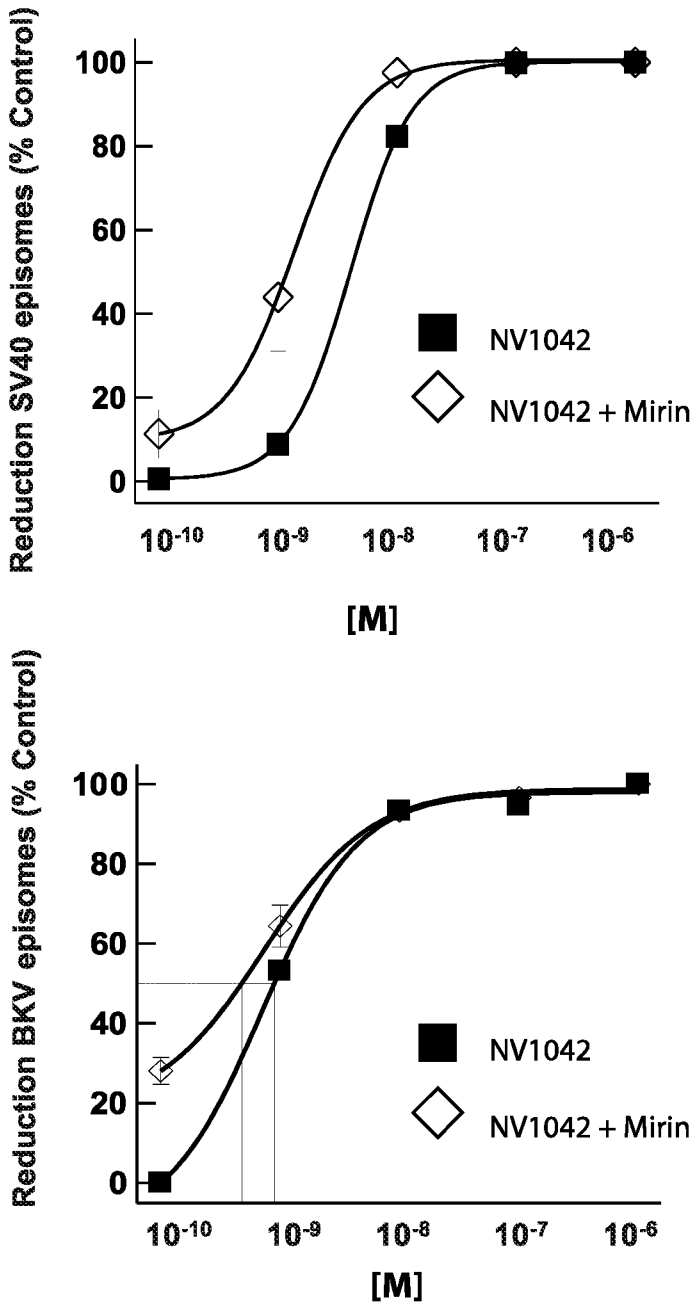

Reduction SV40 episomes (% Control)
100
80
60
40
20
0
NV1042
NV1042 + Mirin
10-10
10-9
10-8
10-7
10-6
[M]
Reduction BKV episomes (% Control)
100
80
60
40
20
0
NV1042
NV1042 + Mirin
10-10
10-9
10-8
10-7
10-6
[M]

Figure 7

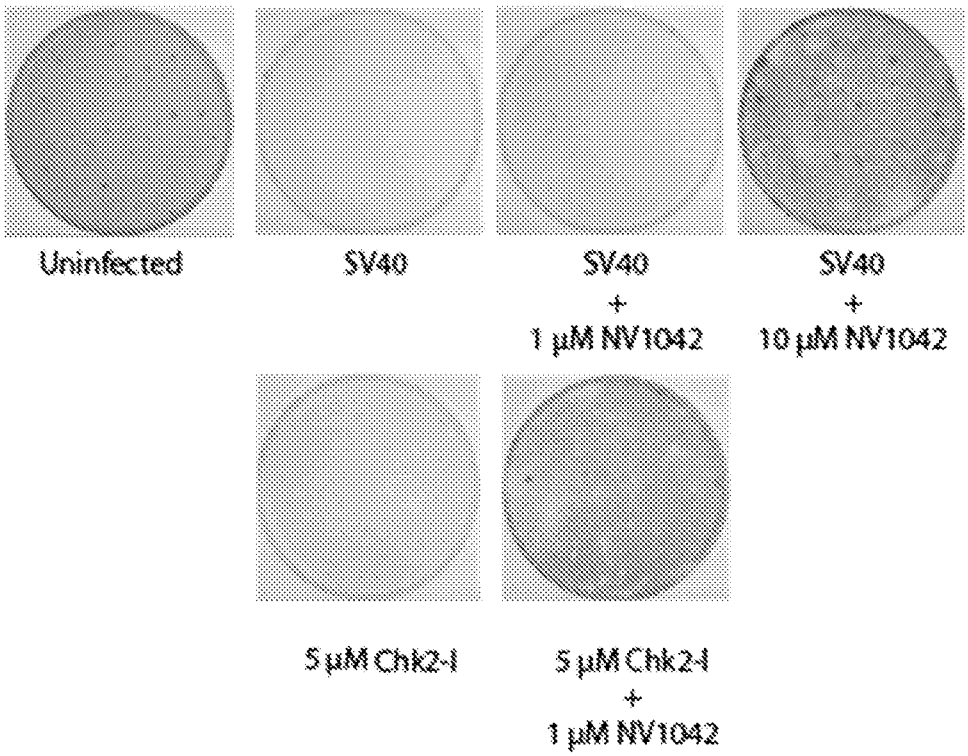
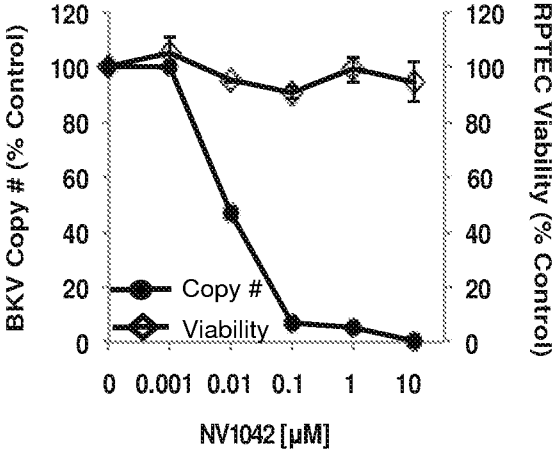
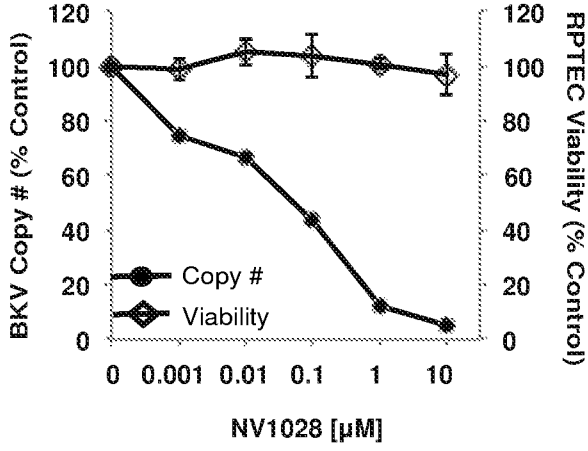
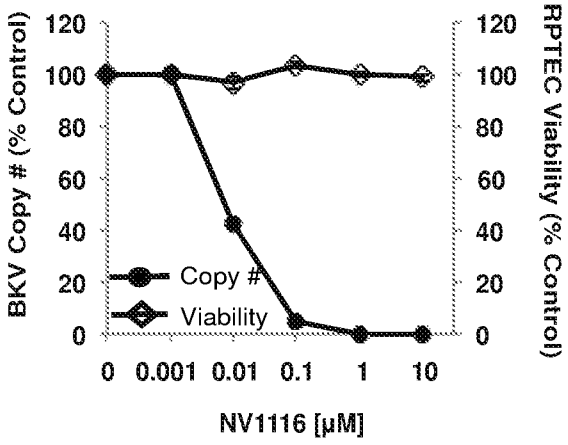
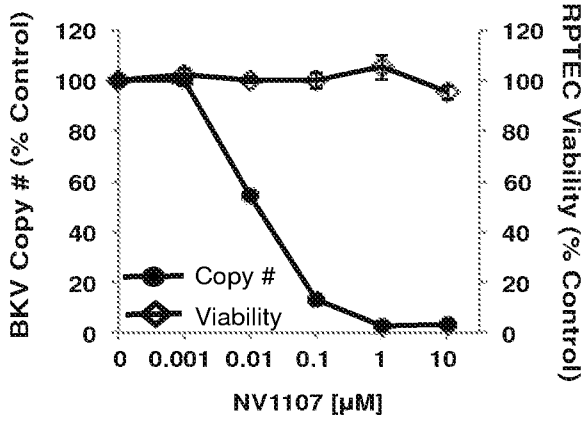

Uninfected
SV40
SV40 + 1 µM NV1042
SV40 + 10 µM NV1042
5 µM Chk2-I
5 µM Chk2-I + 1 µM NV1042

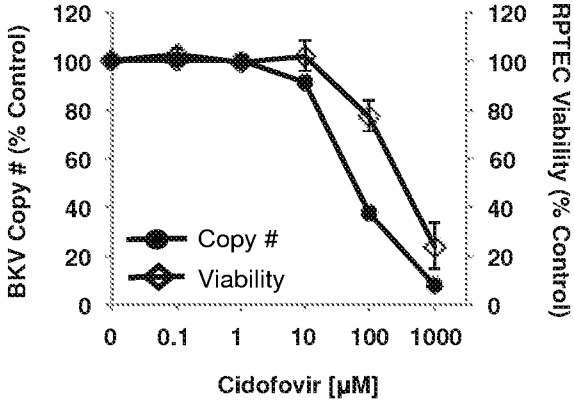
Figure 8

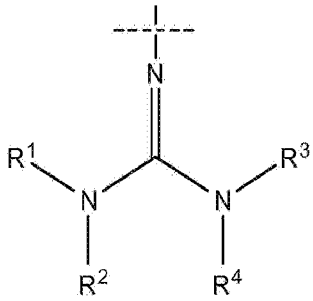

BKV Copy # (% Control)
RPTEC Viability (% Control)
Copy #
Viability
NV1042 [µM]


BKV Copy # (% Control)
RPTEC Viability (% Control)
Copy #
Viability
NV1028 [µM]

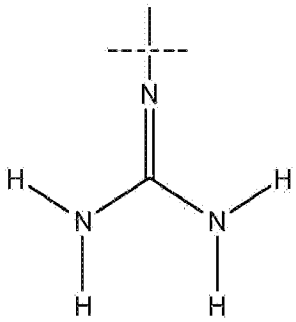

BKV Copy # (% Control)
RPTEC Viability (% Control)
Copy #
Viability
NV1116 [µM]


BKV Copy # (% Control)
RPTEC Viability (% Control)
Copy #
Viability
NV1107 [µM]

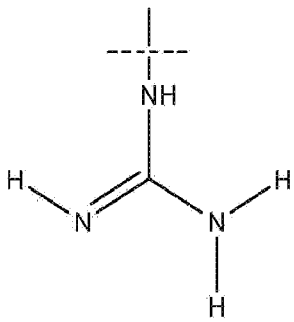

BKV Copy # (% Control)
RPTEC Viability (% Control)
Copy #
Viability
Cidofovir [µM]



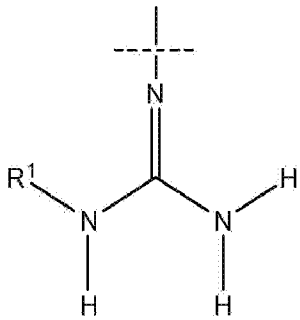
Guanidinyl Radical



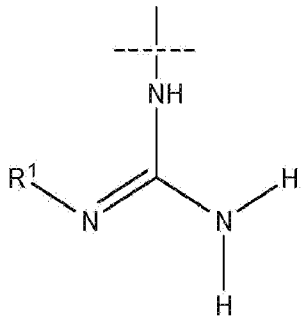
$R^{1-4} = H$

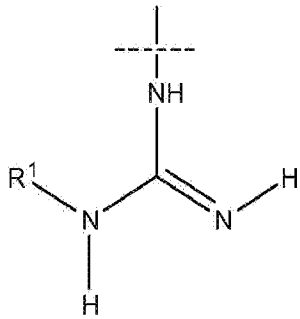


Tautomer

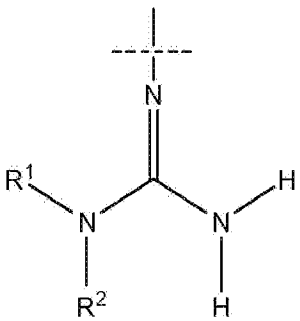


$R^{1} =$ alkyl, aryl, aralkyl
$R^{2-4} = H$

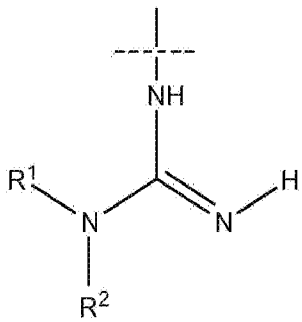




Tautomers

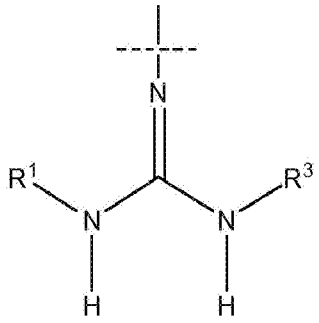


$R^{1,2} =$ alkyl, aryl, aralkyl
$R^{3,4} = H$

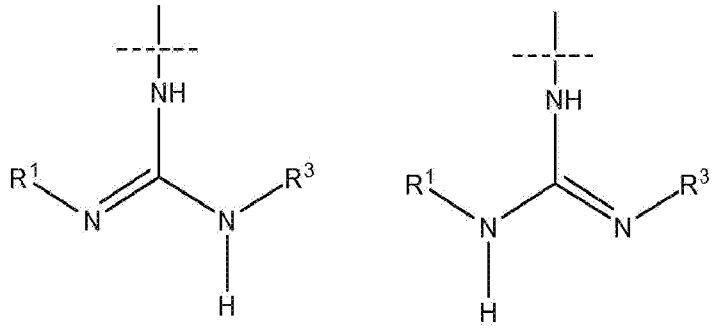


Tautomer

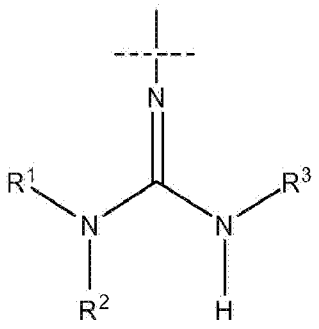
Figure 9A



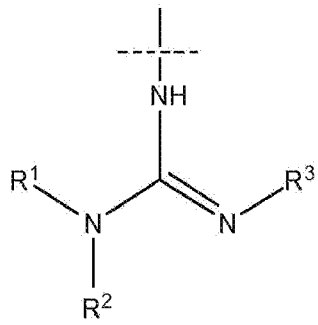
$R^{1,3}$ = alkyl, aryl, aralkyl
$R^{2,4}$ = H



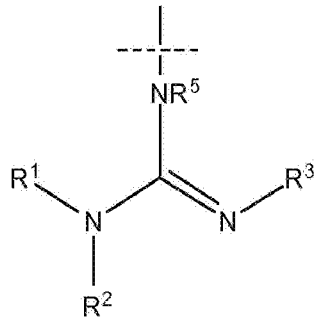
Tautomers (equivalent when $R^1 = R^3$)



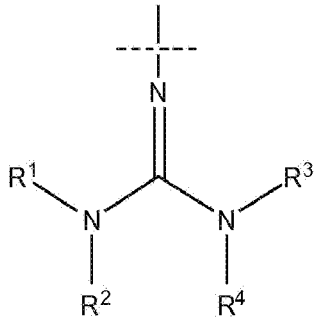
$R^{1-3,5}$ = alkyl, aryl, aralkyl
R^4 = H



Tautomer



Alkylated analog
of tautomer to the left



R^{1-4} = alkyl, aryl, aralkyl

Figure 9B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/043781

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D403/14 C07K5/06 C08G18/60 A61K31/415 A61P31/20
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D C07K C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/130616 A2 (NANOVIR LLC [US]; BASHKIN JAMES K [US]; KOELLER KEVIN J [US]; EDWARDS) 15 November 2007 (2007-11-15) the whole document	1-8
X	WO 2013/055825 A2 (NANOVIR LLC [US]; BASHKIN JAMES K [US]; EDWARDS TERRI G [US]; FISHER C) 18 April 2013 (2013-04-18) the whole document	1-8
X	US 2012/225809 A1 (BASHKIN JAMES K [US] ET AL) 6 September 2012 (2012-09-06) the whole document	1-8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2015	13/10/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Fanni, Stefano

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/043781

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007130616 A2	15-11-2007	NONE	
WO 2013055825 A2	18-04-2013	AU 2012322835 A1	17-04-2014
		CA 2851516 A1	18-04-2013
		CN 104024247 A	03-09-2014
		EP 2751096 A2	09-07-2014
		HK 1201064 A1	21-08-2015
		JP 2015502330 A	22-01-2015
		KR 20140074996 A	18-06-2014
		US 2013090362 A1	11-04-2013
		WO 2013055825 A2	18-04-2013
US 2012225809 A1	06-09-2012	NONE	