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Diallo et al.(10) **Pub. No.: US 2018/0305345 A1**(43) **Pub. Date: Oct. 25, 2018**(54) **COMPOSITIONS AND METHODS FOR
ENHANCING ONCOLYTIC VIRUS
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(57)

ABSTRACT

Provided are compounds that enhance the efficacy of viruses by increasing spread of the virus in cells, increasing the titer of virus in cells, or increasing the cytotoxicity of virus to cells. Other uses, compositions and methods of using same are also provided.

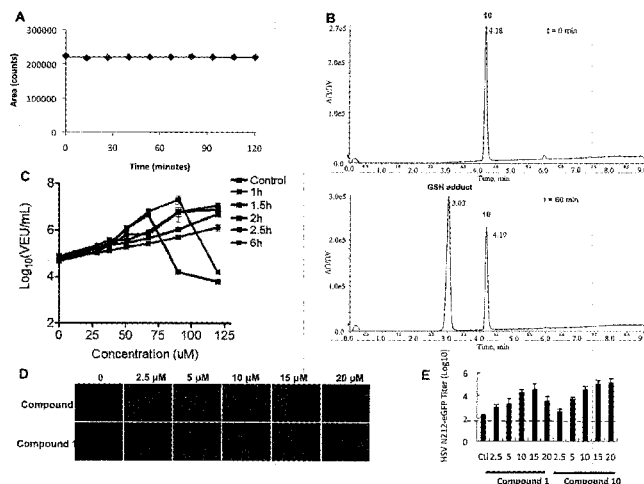


Figure 1

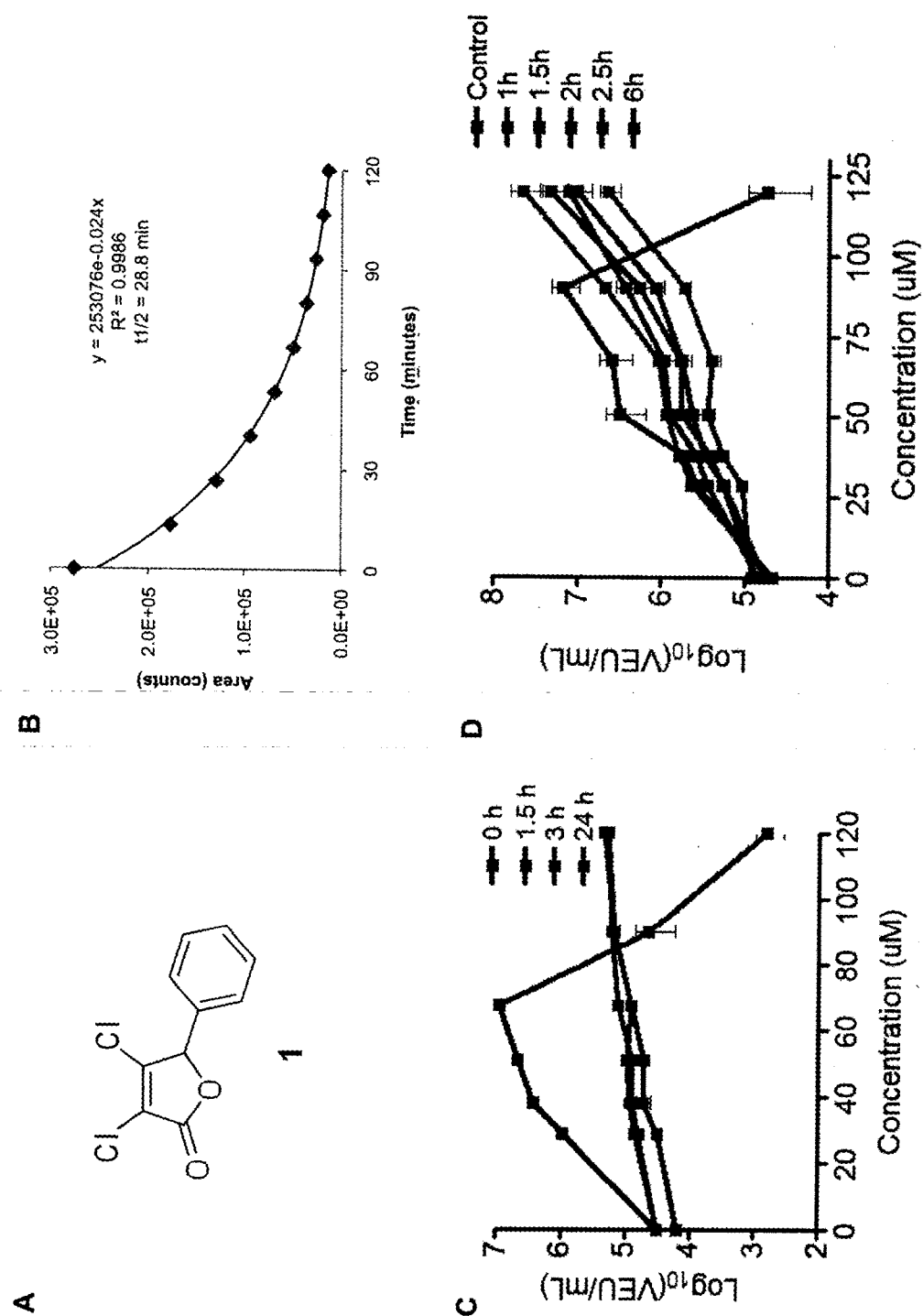


Figure 2

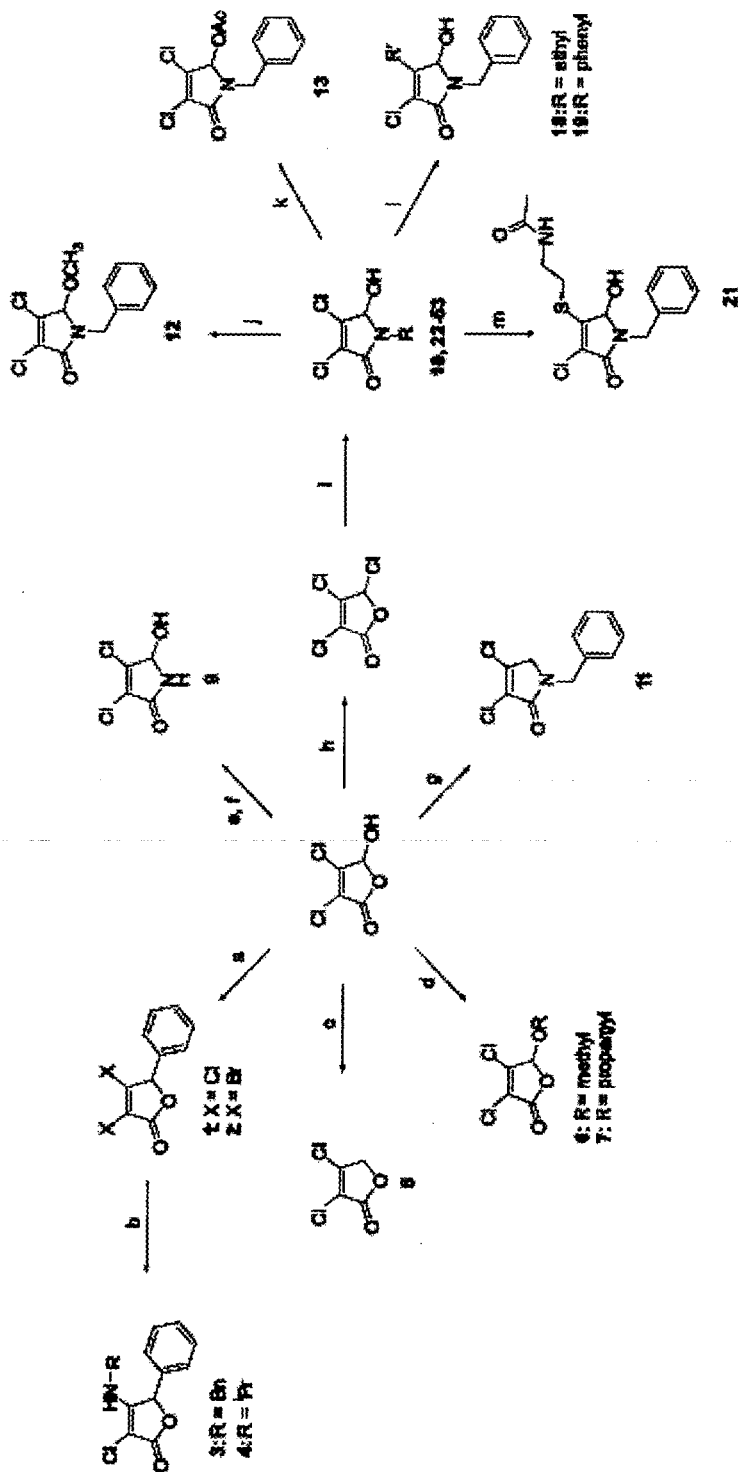


Figure 3

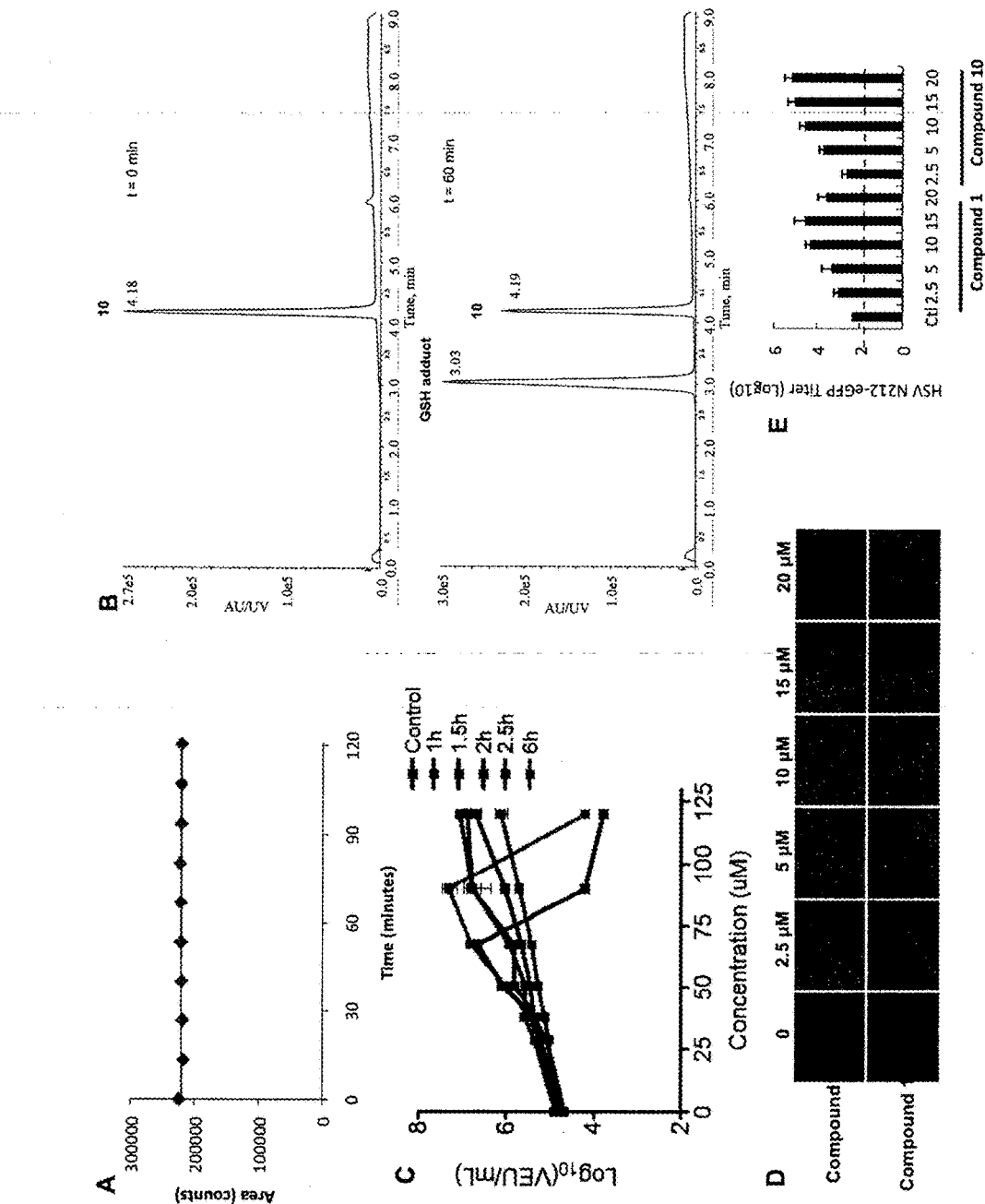


Figure 4

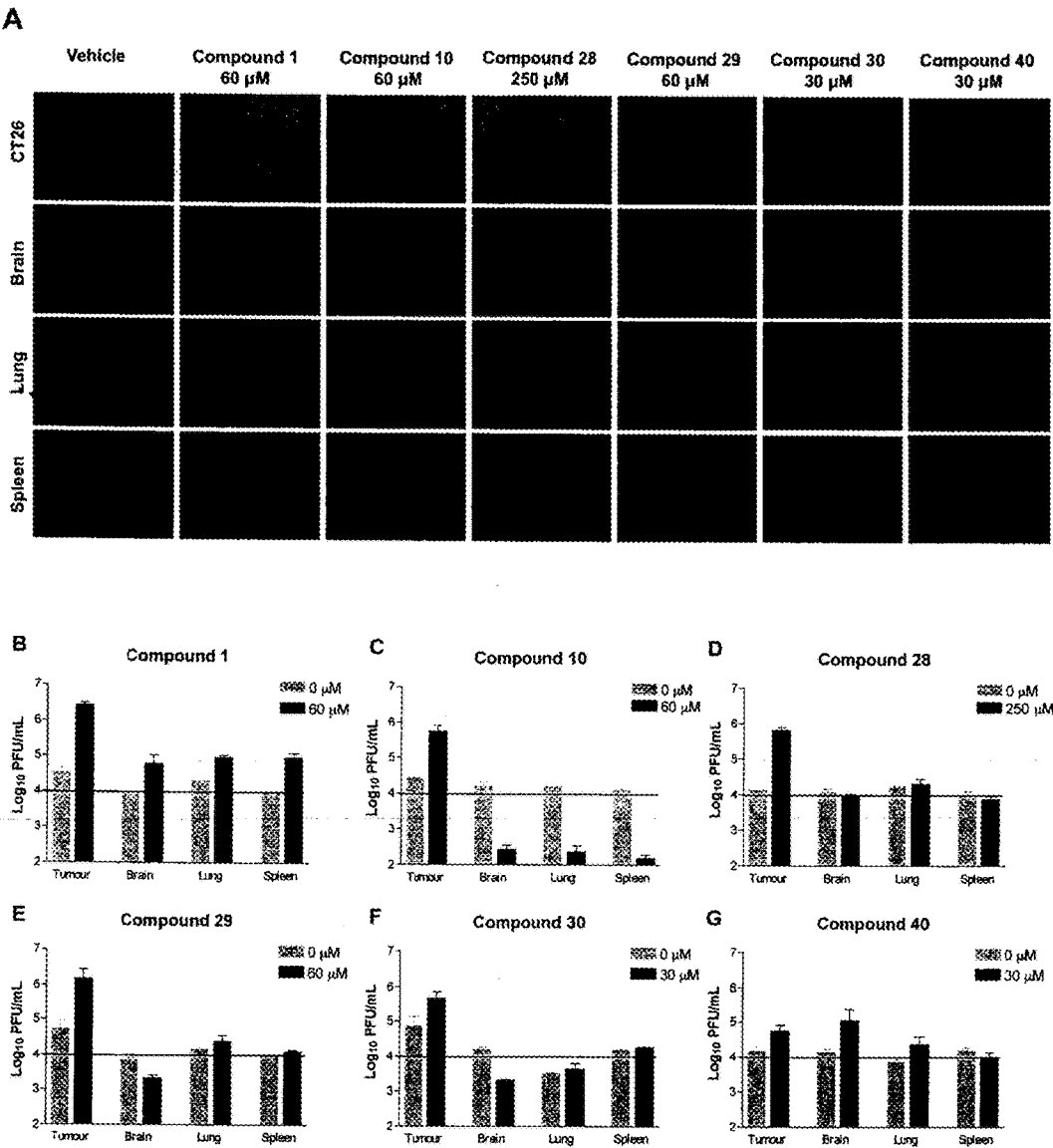


Figure 5

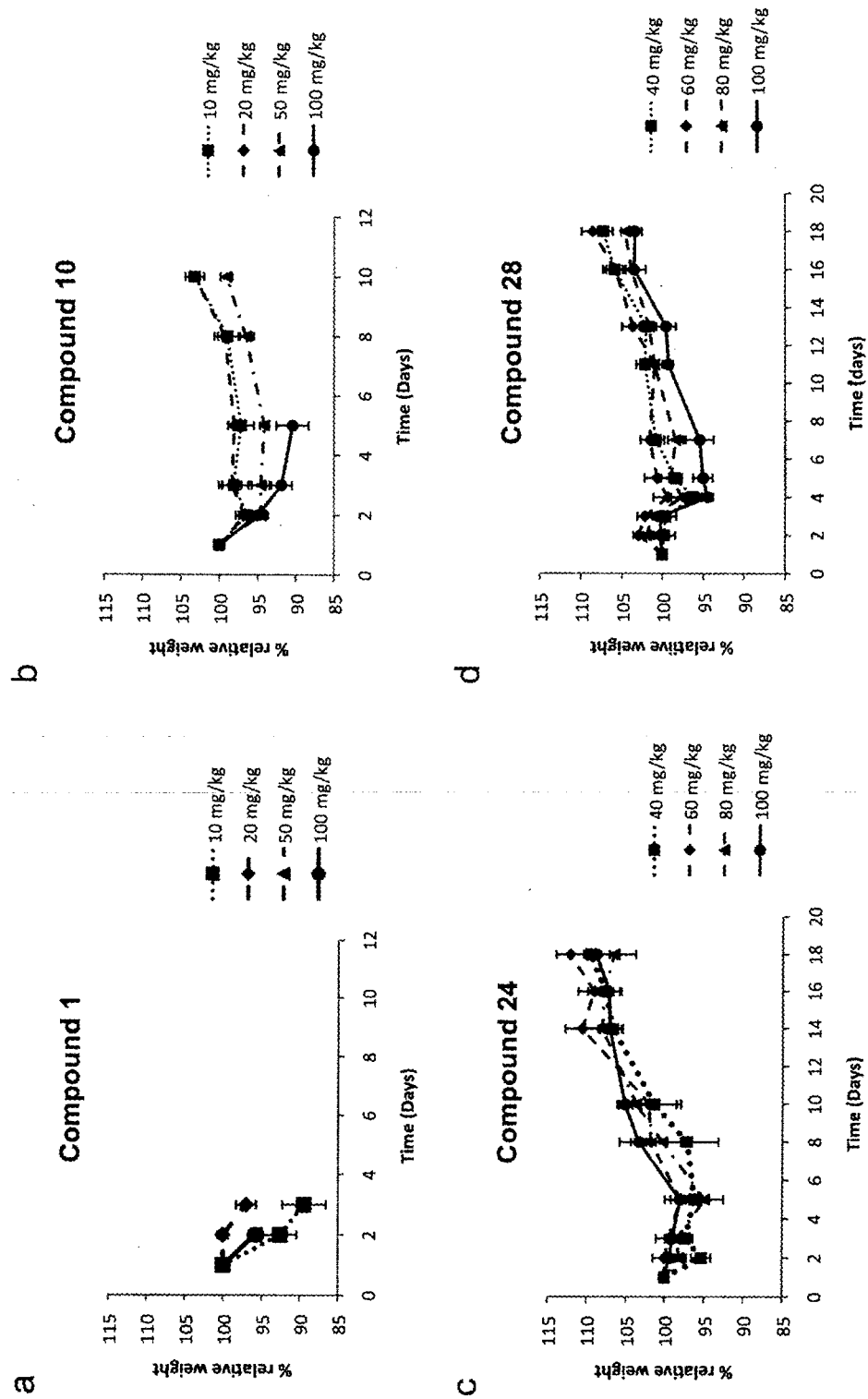


Figure 6

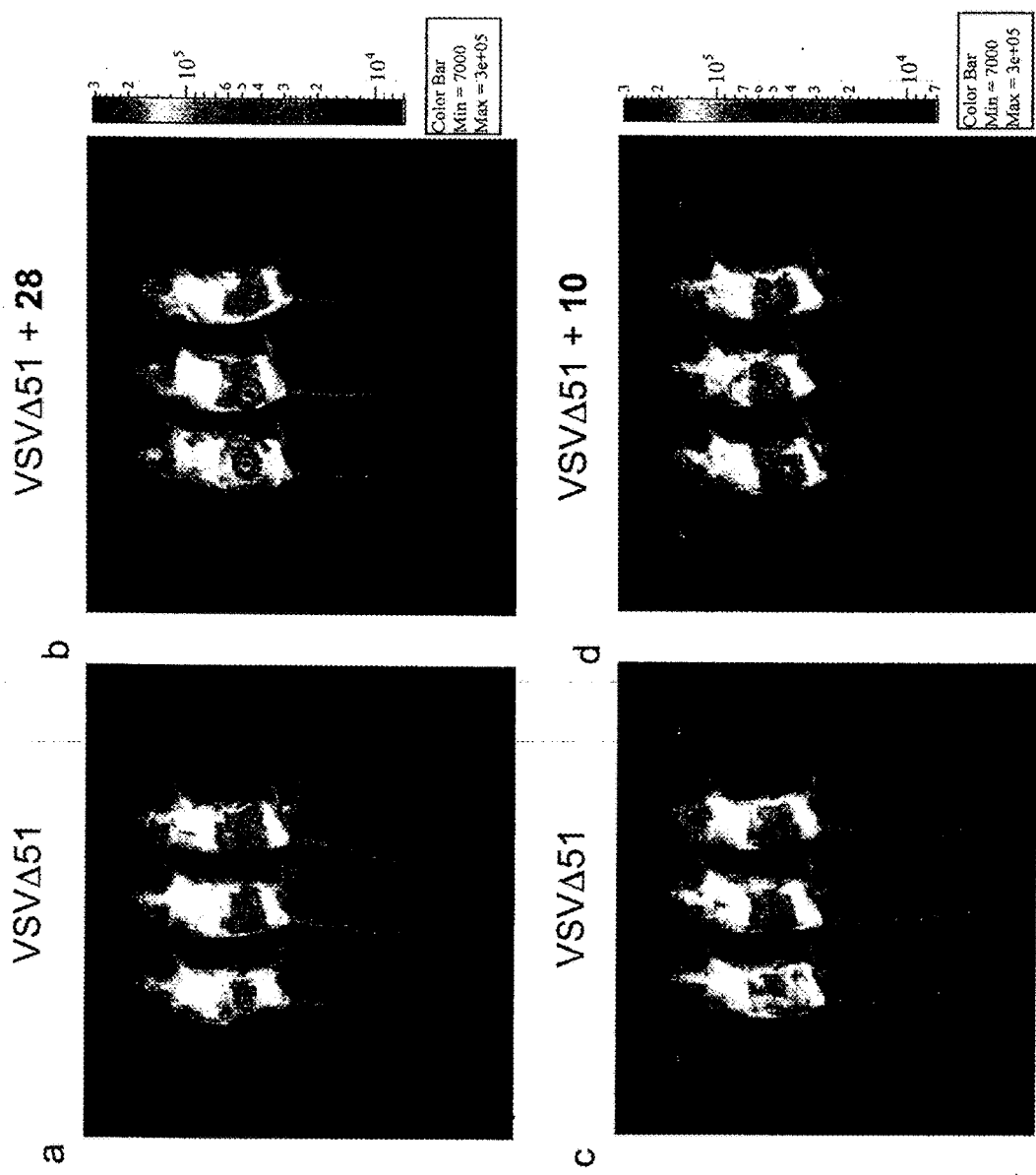


Figure 7

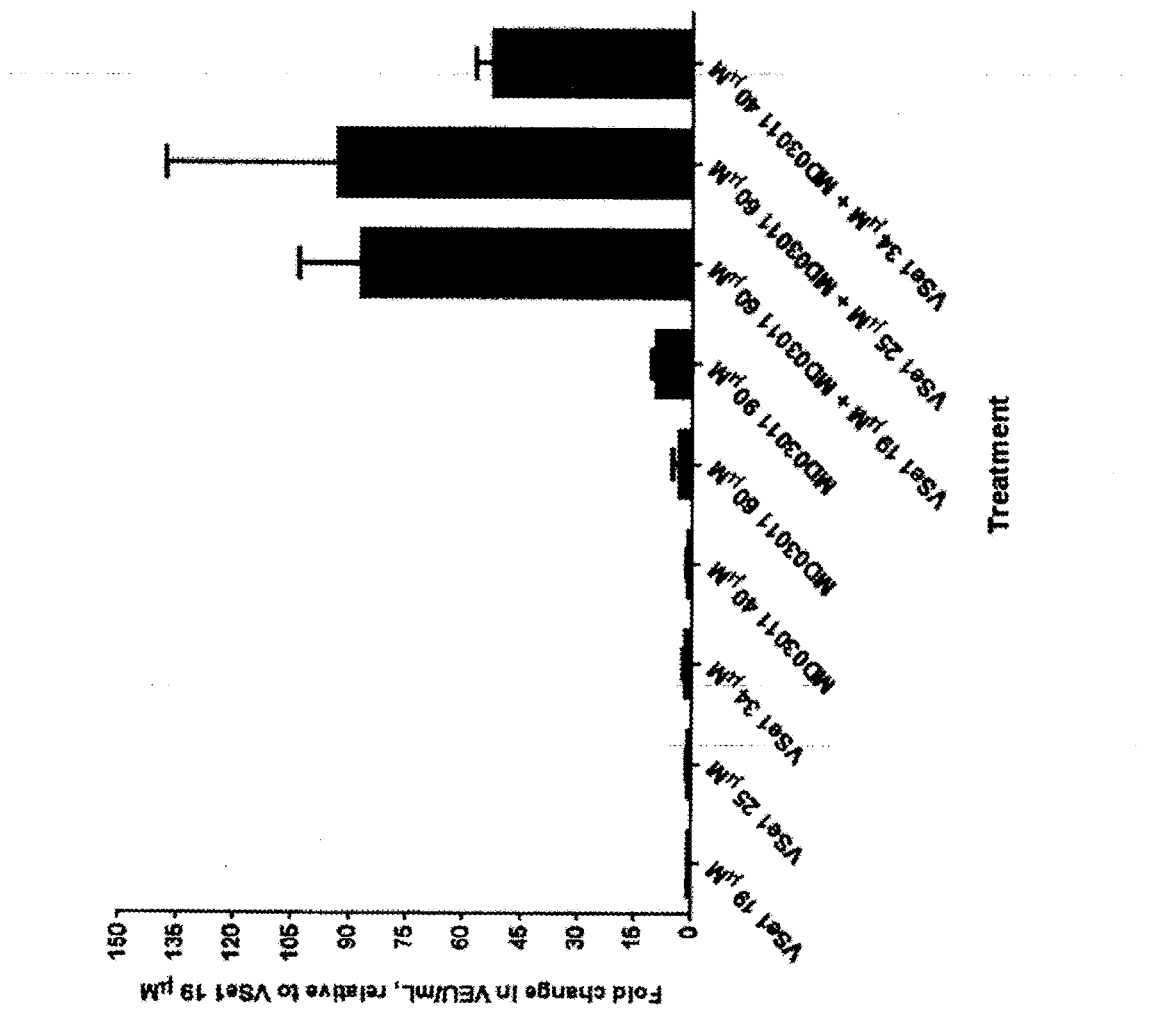


Figure 8

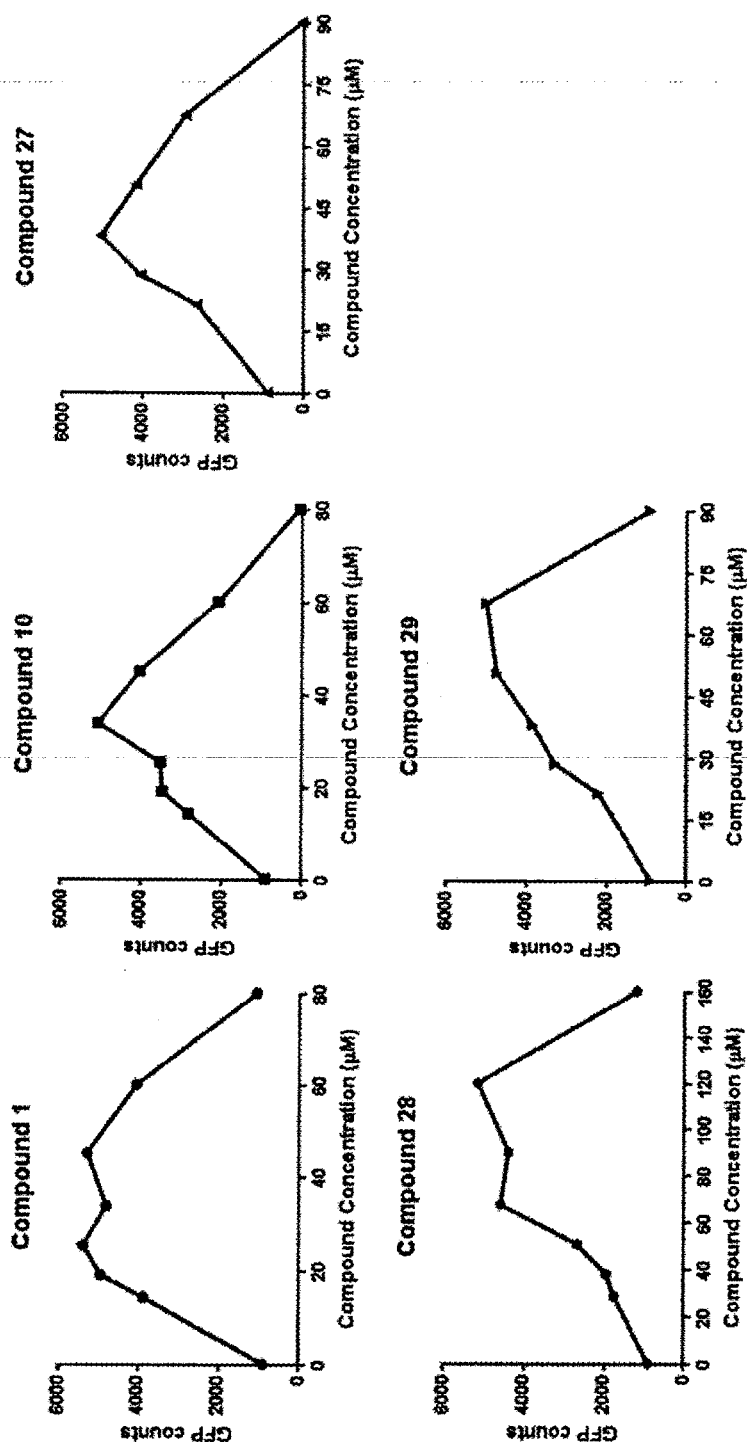


Figure 9

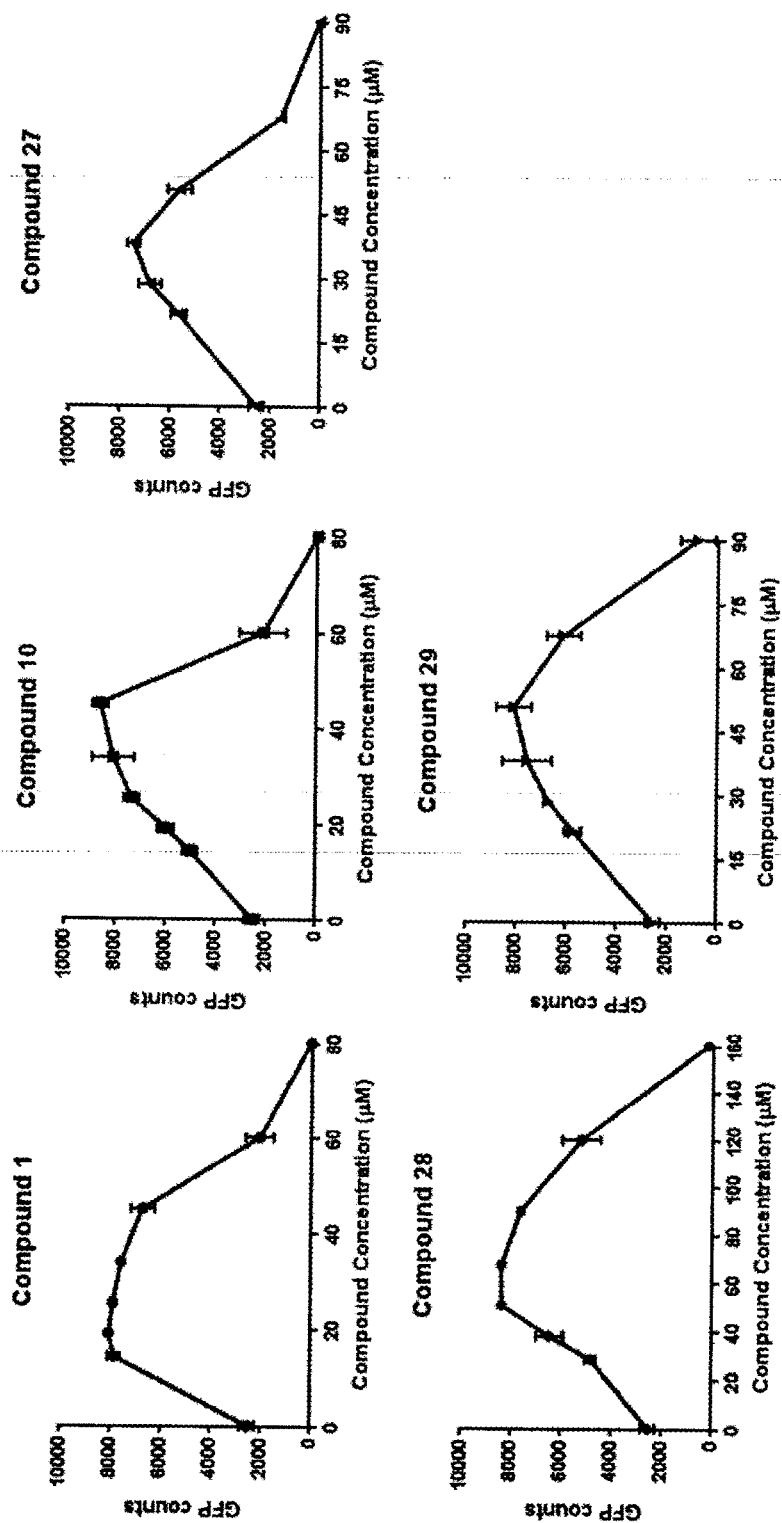
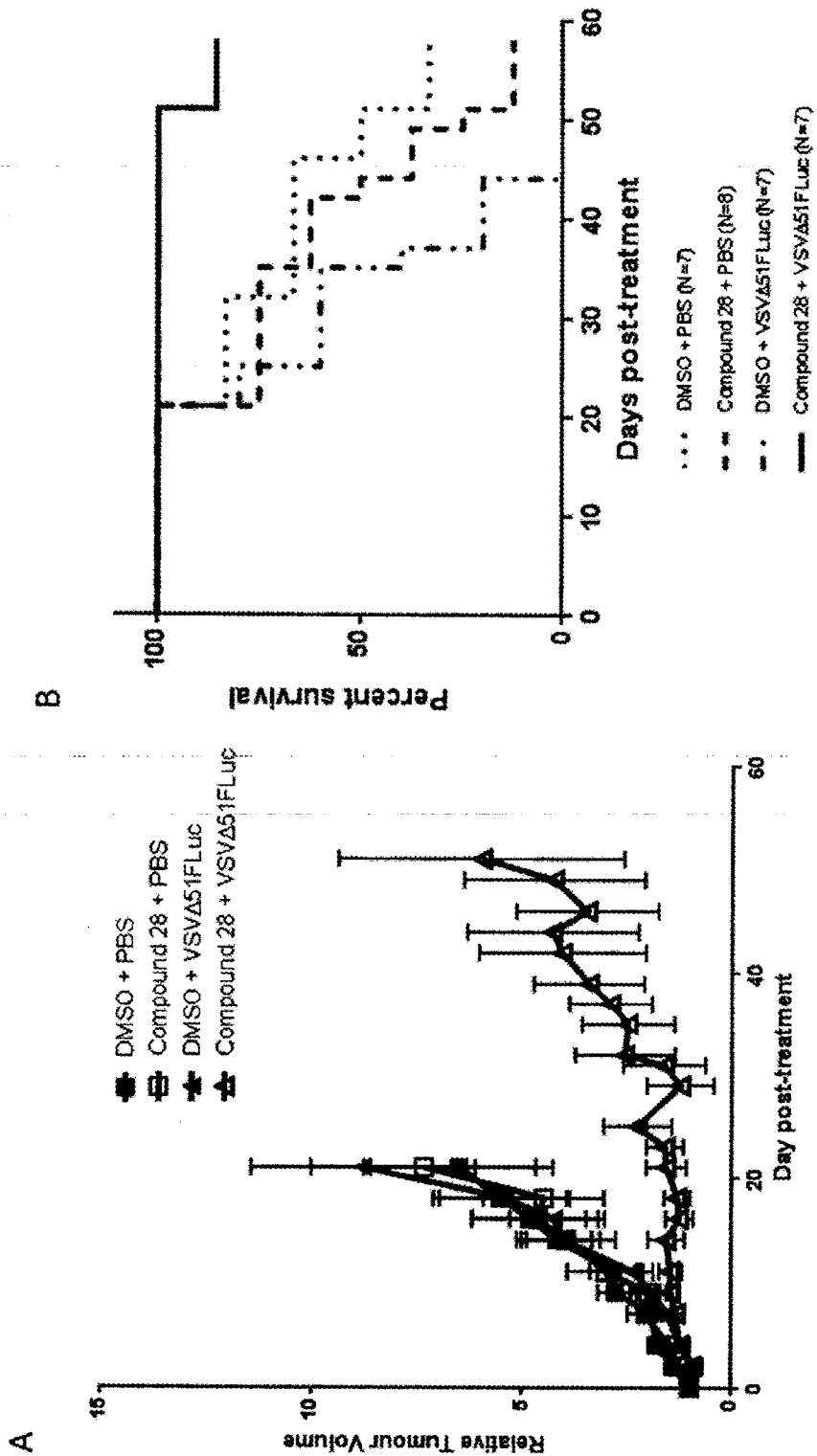


Figure 10



COMPOSITIONS AND METHODS FOR ENHANCING ONCOLYTIC VIRUS EFFICACY

FIELD OF INVENTION

[0001] The present invention relates to compounds, methods, and compositions that enhance viral infection, growth, spread or virus-induced cytotoxicity.

BACKGROUND OF THE INVENTION

[0002] Genetically attenuated viruses form the basis of a growing number of biotechnology and pharmaceutical platforms. Emerging in the field of cancer therapeutics, oncolytic virotherapy has shown significant promise over the last decade. A number of oncolytic viruses (OV) based on a wide range of viral backbones from small RNA viruses (eg. rhabdoviruses), to large DNA viruses (eg. poxviruses, herpesviruses) are currently being evaluated in clinical trials to treat a range of cancer types. Generating substantial excitement for this form of cancer therapy, approval of the first-in-class OV based on herpes-simplex virus-1 (HSV-1) for treatment of melanoma is expected in the next year.

[0003] Oncolytic viruses (OVs) are self-amplifying biotherapeutic agents that have been selected or engineered to preferentially infect and kill cancer cells. When effective, OVs lead to tumor eradication not only by direct lysis of cancer cells but also through downstream generation of anti-cancer immune responses, vascular shutdown, and therapeutic transgene expression. As a basis for their selectivity, OVs exploit cellular defects that are inherent to the cancerous phenotype. This includes dysfunctional anti-viral responses and immune evasion, increased cell proliferation and metabolism, and leaky tumor vasculature. The biological environment ensuing from tumorigenesis is well suited to support the growth of genetically attenuated OVs that are otherwise harmless to normal cells.

[0004] OVs stand to be an attractive therapeutic modality for cancer because of their curative potential and their relatively mild side effects amounting to acute flu-like symptoms. However, heterogeneity in the clinical response to OVs remains a significant hurdle to overcome, as demonstrated in several human clinical trials. This heterogeneity in response may be attributed to factors that impede effective OV delivery and spread within tumors.

[0005] It is well recognized in the OV field that viral engineering or combinations of therapeutic modalities are critical areas that need to be explored to improve efficacy. Recently, a high-throughput small molecule phenotypic screen was conducted to identify compounds that sensitize resistant cancer cells to infection with the rhabdovirus-based OV named VSVA51. VSVA51 is an engineered mutant of vesicular stomatitis virus (VSV) that is highly sensitive to interferon (IFN) and its antiviral effects. Much like HSV-1 and many other OVs, VSVA51 faces a roadblock in tumors that retain effective cellular antiviral responses.

[0006] VSe1 (3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one, FIG. 1a, also referred to as compound 1 herein) emerged as one of the most active hits from this phenotypic screen and was shown to enhance VSVA51 oncolysis in vitro and in vivo, increasing virus output by as much as 1,000-fold preferentially in cancer cells. While the molecular target remains elusive, the compound has been shown to dampen the activation of antiviral responses—most promi-

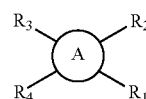
nently the transcriptional response to type I IFN. Given the potential use of VSe1 for co-administration with OVs in humans, the electrophilic nature of VSe1 prompted us to investigate the scaffold to identify active analogues with more favourable physiochemical properties and explore structure-activity relationships. In this study we report the development of a new class of small molecules derived from VSe1 that significantly enhances OV propagation leading to oncolysis in resistant cancers. Other beneficial effects are also demonstrated.

[0007] The prior art associated with this subject area includes the subject matter encompassed by references 1-74 (and citations therein) provided in the reference section of this document. All of the citations are herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0008] The present invention relates to compounds, compositions and methods that enhance viral infection, growth, spread or cytotoxicity.

[0009] In an embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (I):



(I)

[0010] an N-oxide, pharmaceutically acceptable addition salt, quarternary amine or stereochemically isomeric form thereof, wherein:

[0011] A is a 5-membered heterocyclic ring comprising 0 or 1 double bond and 1 heteroatom selected from O, substituted or unsubstituted N;

[0012] R¹ and R⁴ are each independently H, oxo, hydroxyl, alkynyloxy, phenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, or indolyl; and

[0013] R² and R³ are each independently hydrogen, halogen, alkynylamino, isobutylamino, or benzylamino;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0014] In another embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, selected from the group consisting of:

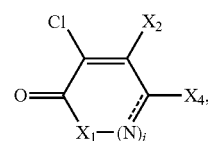
[0015] α,β-dichloro-γ-hydroxy-N-benzyl-crotonic lactam; 3,4-dichloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dibromo-5-prop-2-ynyloxy-5H-furan-2-one; 3-chloro-5-phenyl-4-prop-2-ynylamino-5H-furan-2-one; 3-chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one; 1-benzyl-3,4-dichloro-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-(2-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one; 4-benzylamino-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-prop-2-ynyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5H-furan-2-one;

benzo[1,3]dioxole-5,6-dione; 4,5-dichloro-2H-pyridazin-3-one; 4,5-dichloro-2-phenyl-2H-pyridazin-3-one; 3,4-dichloro-1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-5-(1H-indol-3-yl)-5H-furan-2-one, indole-3-crotonic acid; 3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 3,4-dichloro-5-(3-nitro-phenyl)-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-prop-2-ynyl-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-(2-chloro-benzyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-propyl-1,5-dihydro-pyrrol-2-one; 1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-1-propyl-pyrrole-2,5-dione; 1-benzyl-3,4-dichloro-pyrrole-2,5-dione; 3,4-dichloro-5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one; 1-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; [4-(4-chloro-3-isobutylamino-5-oxo-2,5-dihydro-furan-2-yloxymethyl)-[1,2,3]triazol-1-yl]-acetic acid; [4-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-pyrrol-1-ylmethyl)-[1,2,3]triazol-1-yl]-acetic acid; 3,4-dichloro-5-hydroxy-1-phenethyl-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-morpholinoethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-cyclopropyl-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-mercaptoethyl)-1H-pyrrol-2(5H)-one; 2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethanaminium 2,2,2-trifluoroacetate; 3,4-dichloro-5-hydroxy-1-(3-phenylprop-2-ynyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(trifluoromethyl)benzyl)-1H-pyrrol-2(5H)-one; 1-(biphenyl-4-ylmethyl)-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-nitrobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-methoxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(2-chlorobenzyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 1-benzhydryl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(naphthalen-1-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(1-phenylethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-3-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-4-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(furan-2-ylmethyl)-5-hydroxy-1H-pyrrol-2(5H)-one; N-(2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide; (3aS)-2,3-dichloro-5-phenyl-4,5-dihydropyrrolo[1,2-a]quinolin-1(3aH)-one; 3,4-diiodo-2-phenyl-2,5-dihydrofuran; D-Gluconamide, N-octyl; (S)-11-amino-4,7,10,14-tetraoxo-15-((2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-3,6,9,13-tetraazapentadecan-1-oic acid; 1-allyl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-hydroxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(thiophen-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(methylsulfonyl)benzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-((4,5-dimethylloxazol-2-yl)methyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(3,4,5-trifluorobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-

hydroxy-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one; 4,5-dichloro-2-(2,2,2-trifluoroethyl)pyridazin-3(2H)-one; 4,5-dichloro-2-cyclohexylpyridazin-3(2H)-one; methyl 2-(4-((3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate; 4,5-dichloro-2-o-tolylpyridazin-3(2H)-one; 4,5-dichloro-2-(2-(dimethylamino)ethyl)pyridazin-3(2H)-one hydrochloride; and 4,5-dichloro-2-(4-fluorophenyl)pyridazin-3(2H)-one or any compound or group of compounds defined in Table 1;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0016] In another embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (II):



(II)

or

[0017] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0018] X_1 is a heteroatom such as O, NH, or substituted N;

[0019] X_2 is halogen (such as, for example, Cl), or NHX_3 , wherein X_3 is a substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl;

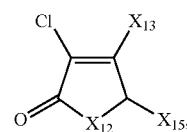
[0020] i is 0 when X_1 is O, or 0 or 1 when X_1 is NH or substituted N;

[0021] --- represents a double bond which is present when i is 1, and absent when i is 0 such that X_1 is directly bonded to the X_4 -bearing carbon through a single bond when i is 0; and

[0022] X_4 is H, OH, =O, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl (such as, for example, substituted or unsubstituted phenyl), or OX_{10} , wherein X_{10} is H, linear or branched substituted or unsubstituted alkyl, alkenyl, alkynyl, or acyl (for example, X_{10} may be acetyl, methyl, or $-CH_2-C\equiv CH$);

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0023] In yet another embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (VIII):



(VIII)

[0024] or

[0025] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

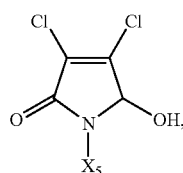
[0026] X_{12} is O, NH, or substituted N;

[0027] X_{13} is halogen such as Cl, or NHX_{14} , wherein X_{14} is a substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl; and

[0028] X_{15} is H, OH, $=O$, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl such as substituted or unsubstituted phenyl, or OX_{16} , wherein X_{16} is H, linear or branched substituted or unsubstituted alkyl, alkenyl, alkynyl, or acyl, or X_{16} is acetyl, methyl, or $-CH_2-C\equiv CH$;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0029] In another embodiment of any one of the method or methods described herein, the compound, or at least one of the compounds of the combination of compounds, may be defined by formula (III):



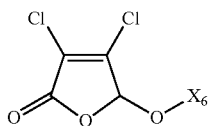
(III)

or

[0030] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0031] X_5 is H, substituted or unsubstituted linear or branched C_1 - C_{12} alkyl, alkenyl, or alkynyl, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl, substituted or unsubstituted cycloalkyl or heterocycloalkyl, or wherein X_5 is substituted or unsubstituted alkynyloxy, phenyl, alkylphenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, naphthalenyl, substituted naphthalenyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted furanyl or thiofuranyl, thiophenyl, sulfonobenzyl, methylsulfonobenzyl, pyrrolyl, substituted or unsubstituted morpholine, cycloalkyl, alkylthiol, substituted or unsubstituted alkyamine, or substituted or unsubstituted oxazoline.

[0032] In yet another embodiment of any one of the method or methods described herein, the compound, or at least one of the compounds of the combination of compounds, may be defined by formula (IV):



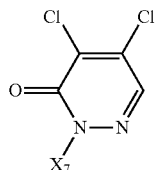
(IV)

or

a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0033] wherein X_6 is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or wherein X_6 is substituted or unsubstituted methyl, alkyl triazolyl, acetyl, or $-CH_2-C\equiv CH$.

[0034] In yet another embodiment of any one of the method or methods described herein, the compound, or at least one of the compounds of the combination of compounds, may be defined by formula (V):



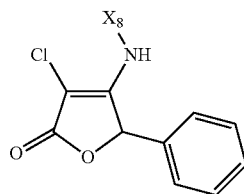
(V)

or

[0035] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0036] X_7 is H, substituted or unsubstituted aryl or heteroaryl, substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted cycloalkyl. For example, X_7 may be substituted or unsubstituted alkylamine, or substituted or unsubstituted phenyl.

[0037] In yet another embodiment of any one of the method or methods described herein, the compound, or at least one of the compounds of the combination of compounds, may be defined by formula (VI):



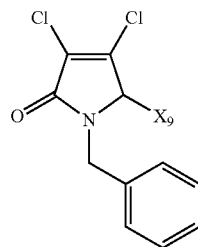
(VI)

or

[0038] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0039] wherein X_8 is substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl, or X_8 is substituted or unsubstituted benzyl.

[0040] In yet another embodiment of any one of the method or methods described herein, the compound, or at least one of the compounds of the combination of compounds, may be defined by formula (VII):



(VII)

or

[0041] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0042] wherein X_9 is H, OH, OX_{11} , or $=O$, wherein X_{11} is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or X_{11} is acetyl, methyl, or $-CH_2-C\equiv CH$.

[0043] In still another embodiment of any one of the method or methods described herein, the compound or combination of compounds may be present in a composition comprising the compound(s) and a carrier, diluent or excipient.

[0044] In another embodiment, there is provided herein a composition comprising one or more of the compounds having a structure as defined in any of the method or methods as described above, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more cancer cells, c) a carrier, diluent or excipient, d) a pharmaceutically acceptable carrier, diluent or excipient, e) non-cancer cells; f) cell culture media; g) one or more cancer therapeutics; or any combination of a)-g).

[0045] In yet another embodiment, there is provided herein a kit comprising one or more of the compounds having a structure as defined in any of the method or methods as described above, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more cancer cells, c) a pharmaceutically acceptable carrier, diluent or excipient, d) non-cancer cells; e) cell culture media; f) one or more cancer therapeutics, g) a cell culture plate or multi-well dish; h) an apparatus to deliver the compound to a cell, medium or to a subject; i) instructions for using the compound or any component in the kit, j) a carrier diluent or excipient, or any combination of a)-j).

[0046] In another embodiment, the cells may be cancer cells in vivo, or in vitro.

[0047] In a further embodiment, the in vivo cancer cells may be from a mammalian subject.

[0048] In still a further embodiment, the mammalian subject may be a human subject.

[0049] In yet another embodiment, there is provided herein a method of increasing the oncolytic activity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, having a structure as defined in any of the method or methods as described above to said cancer or tumor cells prior to, concurrently with or after the oncolytic virus.

[0050] In another embodiment, the cancer cells may be in vivo, or in vitro.

[0051] In still another embodiment, the in vivo cancer cells may be from a mammalian subject.

[0052] In yet another embodiment, the mammalian subject may be a human subject.

[0053] In another embodiment, there is provided herein a compound having a structure as defined in any of the method or methods as described above. In still another embodiment, the compound may be for use as a viral sensitizer for sensitizing a cancer or tumor cell to an oncolytic virus.

[0054] In an embodiment, there is provided herein a compound having a structure as defined in any of the method or methods as described above, for use as a viral sensitizer to enhance or increase oncolytic virus activity in cancer or tumor cells.

[0055] In a further embodiment, the compound may be for enhancing or increasing oncolytic virus titer in cancer or tumor cells.

[0056] In another embodiment, the compound may be for enhancing or increasing cytotoxicity of the oncolytic virus in cancer or tumor cells.

[0057] In another embodiment, there is provided herein a compound having a structure as defined in any of the method or methods described above, for use in enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells.

[0058] In another embodiment, there is provided herein a use of a compound having a structure as defined in any of the method or methods described above for enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells.

[0059] In another embodiment, there is provided herein a use of a compound having a structure as defined in any of the method or methods described above in the manufacture of a medicament for enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells.

[0060] In a further embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a degradation half-life greater than 20, 40, 60, 80, 100, 120, 240, 360 minutes or more in phosphate buffer at pH 7.4.

[0061] In another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a half-life greater than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400 minutes or more in a glutathione stability assay.

[0062] In still another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, exhibits a lower LD50 in the presence of an oncolytic virus than in its absence.

[0063] In a further embodiment, the difference in LD50 may be 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μ M or more in the presence of an oncolytic virus compared to its absence.

[0064] In yet another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, or greater, or any range bounded at a lower end by any one of these values, any range bounded at an upper end by any one of these values, or any range falling between any two of these values, when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one. In one embodiment, for example, the compound may be a compound which exhibits a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is greater than or equal to ($\geq$) about 0.01 when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one.

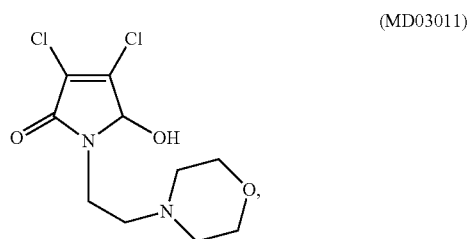
[0065] In still another embodiment, a compound as described above may be a compound for which greater than about 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 90%, or greater, or any range bounded at a lower end by any one of these values, any range bounded at an upper end by any one of these values, or any range falling between any two of these values, of the compound remains after 3 hour incubation at 37° C. in aqueous, protein-rich Balb/c mouse plasma buffered 1:1 with pH 7.4 phosphate buffered saline (PBS). For example, a compound as described above may be a compound for which greater than or equal to ($\geq$) about 0.5% of the compound remains after 3 hour incubation at 37° C. in aqueous, protein-rich Balb/c mouse plasma buffered 1:1 with pH 7.4 phosphate buffered saline (PBS).

[0066] In yet another embodiment, a compound as described above may be a compound having an LD₅₀ in the presence of virus which is greater than or equal to ($\geq$) about 1 μ m, 5 μ m, 10 μ m, 15 μ m, 20 μ m, 25 μ m, 30 μ m, 35 μ m, 40 μ m, 45 μ m, 50 μ m, 55 μ m, 60 μ m, 65 μ m, 70 μ m, 75 μ m, 80 μ m, 85 μ m, 90 μ m, 95 μ m, 100 μ m, or 105 μ m, or 110 μ m, or any range falling between any two of these values, less than (<) the LD₅₀ of the same compound in the absence of virus as determined in, for example, 786-0 cells where the virus is VSV.

[0067] In still another embodiment of any of the method or methods described above, the method may further comprise administration of an anticancer agent or cancer therapeutic.

[0068] In another embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering VSe1 and MD03011,



to said cells prior to, after, or concurrently with infection of the cells with the virus.

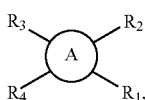
[0069] In an embodiment of any of the method or methods above, the oncolytic virus may be any suitable oncolytic virus known in the art which preferentially infects and lyses cancer or tumor cells as compared to non-cancer or normal cells. Examples of oncolytic viruses known in the art which may be employed herein may include, without limitation, reovirus, newcastle disease virus, adenovirus, herpes virus, polio virus, mumps virus, measles virus, influenza virus, vaccinia virus, rhabdoviruses such as vesicular stomatitis virus and derivatives/variants thereof. In a preferred embodiment, the virus may be a Vesicular stomatitis virus (VSV), or a related rhabdovirus variant/derivative thereof (such as, for example, a maraba virus such as MG1), for example, selected under specific growth conditions, one that has been subjected to a range of selection pressures, one that has been genetically modified using recombinant techniques known within the art, or a combination thereof. In another preferred embodiment, the virus may be VSVΔ51 (Stojdl et al., VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents., Cancer Cell. 2003 October; 4(4):263-75, herein incorpo-

rated by reference). Other derivatives or variants may be based on viruses such as Maraba (MG-1, for example), Rabies, Rotavirus, Influenza, Hepatitis A, Mumps, Measles, Rubella, Herpesvirus, Reovirus, Parvovirus, Dengue Virus, Cickungunya Virus, Vaccinia virus, Modified Vaccinia Ankara, Respiratory Syncytial Virus, Varicella, LCMV, HSV-1, HSV-2, adenovirus, adeno-associated virus, lentivirus, or replicating retrovirus, for example.

[0070] In another embodiment of any of the method or methods above, the one or more types of cancer or tumor cells may be cancer or tumor cells in vitro or in vivo from any cell, cell line, tissue or organism, for example, but not limited to human, rat, mouse, cat, dog, pig, primate, horse and the like. In a preferred embodiment, the one or more cancer or tumor cells comprise human cancer or tumor cells, for example, but not limited to lymphoblastic leukemia, myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytoma, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, malignant fibrous histiocytoma, brain stem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, craniopharyngioma, ependymoblastoma, medulloblastoma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma, visual pathway and hypothalamic glioma, spinal cord tumors, breast cancer, bronchial tumors, Burkitt lymphoma, carcinoid tumor, central nervous system lymphoma, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-Cell lymphoma, embryonal tumors, endometrial cancer, ependymoblastoma, ependymoma, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), gastrointestinal stromal cell tumor, germ cell tumors, extracranial, extragonadal, ovarian, gestational trophoblastic tumor, glioma, hairy cell leukemia, head and neck cancer, hepatocellular (Liver) cancer, histiocytosis, Langerhans cell cancer, Hodgkin lymphoma, hypopharyngeal cancer, islet cell tumors, Kaposi sarcoma, kidney cancer, laryngeal cancer, lymphocytic leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, non-small cell lung cancer, small cell lung cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, malignant fibrous histiocytoma of bone and osteosarcoma, medulloblastoma, medulloepithelioma, melanoma, intraocular melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal parenchymal tumors, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter cancer, transitional cell cancer, respiratory tract carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, uterine sarcoma, skin

cancer, Merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (Gastric) cancer, supratentorial primitive neuroectodermal tumors, T-Cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, trophoblastic tumor, urethral cancer, uterine cancer, endometrial cancer, uterine sarcoma, vaginal cancer, vulvar cancer, or Wilms tumor. However, the compounds and compositions described herein possible may be used to treat other cancers or tumor in vivo or in vitro.

[0071] According to the present invention there is provided a compound or group of compounds defined by formula (I)



an N-oxide, pharmaceutically acceptable addition salt, quarternary amine or stereochemically isomeric form thereof, wherein A is a 5-membered heterocyclic ring comprising 0 or 1 double bond and 1 heteroatom selected from O, substituted or unsubstituted N, R₁ and R₄ are each independently H, oxo, hydroxyl, alkynyloxy, phenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, or indolyl and R₂ and R₃ are each independently hydrogen, halogen, alkynylamino, isobutylamino, or benzylamino.

[0072] Also provided is one or more compounds or group of compounds selected from the group consisting of α,β-dichloro-γ-hydroxy-N-benzyl-crotonic lactam; 3,4-dichloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dibromo-5-prop-2-ynyloxy-5H-furan-2-one; 3-chloro-5-phenyl-4-prop-2-ynyloxy-5H-furan-2-one; 3-chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one; 1-benzyl-3,4-dichloro-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-(2-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one; 4-benzylamino-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-prop-2-ynyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5H-furan-2-one; benzo[1,3]dioxole-5,6-dione; 4,5-dichloro-2H-pyridazin-3-one; 4,5-dichloro-2-phenyl-2H-pyridazin-3-one; 3,4-dichloro-1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-5-(1H-indol-3-yl)-5H-furan-2-one, indole-3-crotonic acid; 3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 3,4-dichloro-5-(3-nitrophenyl)-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-prop-2-ynyl-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-(2-chloro-benzyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-propyl-1,5-dihydro-pyrrol-2-one; 1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-1-propyl-pyrrole-2,5-dione; 1-benzyl-3,4-dichloro-pyrrole-2,5-dione; 3,4-dichloro-5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one; 1-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; [4-(4-chloro-3-isobutylamino-5-oxo-2,5-dihydro-furan-2-yloxymethyl)-[1,2,3]triazol-1-yl]-acetic acid; [4-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-pyrrol-1-ylmethyl)-[1,2,3]triazol-1-yl]-acetic acid; 3,4-dichloro-5-hydroxy-1-phenethyl-1H-pyrrol-

2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-morpholinoethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-cyclopropyl-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-mercaptoethyl)-1H-pyrrol-2(5H)-one; 2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethanaminium 2,2,2-trifluoroacetate; 3,4-dichloro-5-hydroxy-1-(3-phenylprop-2-ynyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(trifluoromethyl)benzyl)-1H-pyrrol-2(5H)-one; 1-(biphenyl-4-ylmethyl)-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-nitrobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-methoxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(2-chlorobenzyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 1-benzhydryl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(naphthalen-1-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(1-phenylethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-3-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-4-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(furan-2-ylmethyl)-5-hydroxy-1H-pyrrol-2(5H)-one; N-(2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino) naphthalene-1-sulfonamide; (3aS)-2,3-dichloro-5-phenyl-4,5-dihydropyrrolo[1,2-a]quinolin-1(3aH)-one; 3,4-diiodo-2-phenyl-2,5-dihydrofuran; D-Gluconamide, N-octyl; (S)-11-amino-4,7,10,14-tetraoxo-15-((2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-3,6,9,13-tetraazapentadecan-1-oic acid; 1-allyl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-hydroxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(thiophen-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(methylsulfonyl)benzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-((4,5-dimethylloxazol-2-yl)methyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(3,4,5-trifluorobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one; 4,5-dichloro-2-(2,2,2-trifluoroethyl)pyridazin-3(2H)-one; 4,5-dichloro-2-cyclohexylpyridazin-3(2H)-one; methyl 2-(4-((3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate; 4,5-dichloro-2-o-tolylpyridazin-3(2H)-one; 4,5-dichloro-2-(2-(dimethylamino)ethyl)pyridazin-3(2H)-one hydrochloride; and 4,5-dichloro-2-(4-fluorophenyl)pyridazin-3(2H)-one. Any combination of 2, 3, 4, 5 or more compounds from above is also contemplated.

[0073] The present invention also provides a composition comprising the compound(s) as described herein, and an acceptable carrier, diluent or excipient. In a further embodiment, the carrier is a pharmaceutically acceptable carrier.

[0074] Also provided is a composition comprising the compound(s) as described herein, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more type of cells such as, for example, cancer cells, c) a carrier, diluent or excipient, d) a pharmaceutically acceptable carrier, diluent or excipient, e) non-cancer or normal cells; f) cell culture media; g) one or more cancer therapeutics; or any combination of a)-g).

[0075] The present invention further provides a kit comprising the compound(s) as described herein, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more cancer cells, c) a carrier,

diluent or excipient, d) a pharmaceutically acceptable carrier, diluent or excipient, e) non-cancer cells, f) cell culture media, g) one or more cancer therapeutics, h) a cell culture plate or multi-well dish, i) an apparatus to deliver the compound to a cell, medium or to a subject; j) instructions for using the compound or any component in the kit, or any combination of a)-j).

[0076] In one non-limiting embodiment of a composition or kit as described above, the composition or kit may comprise a cell infected with a virus such as, for example, an oncolytic virus.

[0077] Also provided is a method of enhancing or increasing the infection, spread and/or titer, and/or cytotoxicity of a virus in cells comprising, administering the compound(s) as described herein to the cells prior to, after or concurrently with the virus, and culturing the virus and cells to enhance or increase the infection, spread and/or titer, or cytotoxicity of the virus in said cells. Preferably, the cells are cancer cells, tumor cells or cells which have been immortalized. More preferably, the cells are in vivo cancer cells from a mammalian, still more preferably a human subject and the method is practiced in vivo.

[0078] Also provided is a method of enhancing or increasing the oncolytic activity of an oncolytic virus in cancer cells comprising, administering the compound(s) as described herein to the cancer cells or subject prior to, concurrently with or after the oncolytic virus and culturing the oncolytic virus and cancer cells. In a further embodiment, the cancer cells are in vivo cancer cells. In a separate embodiment, the cancer cells are in vitro cancer cells. The cells may be from a mammalian subject, preferably a human subject.

[0079] The present invention also provides a composition comprising the compound as described above, and a carrier, diluent or excipient, more preferably a pharmaceutically acceptable carrier, diluent or excipient.

[0080] Also, the present invention provides a composition comprising the compound as described above and one or more of a) a virus, an attenuated virus, a genetically modified virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus; b) one or more cancer cells; c) a carrier, diluent or excipient; d) a pharmaceutically acceptable carrier, diluent or excipient; e) non-cancer or normal cells, f) cell culture media, g) one or more cancer therapeutics, or any combination of a)-g). The present invention also contemplates embodiments wherein any one or a combination of a-g) are specifically excluded from the composition or kit. Any component or group of components may be excluded if desired.

[0081] In a particular embodiment, which is not meant to be limiting in any manner, there is provided a compound as described above and a medium for growing, culturing or infecting cells with a virus and optionally, one or more cells which are capable of being infected by the virus. In a further embodiment, the cells are cancer cells, tumor cells or immortalized cells.

[0082] Also provided is a kit comprising the compound as described above and a) a virus, preferably an attenuated or genetically modified virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus; b) one or more cancer cells; c) a pharmaceutically acceptable carrier, diluent or excipient; d) non-cancer cells; e) cell culture media; f) one or more cancer therapeutics, g) a cell culture plate or multi-well dish; h) an apparatus to deliver the viral sensitizing compound to a cell, medium or to a subject; i)

instructions for using the viral sensitizing agent; j) a carrier diluent or excipient, or any combination of a)-j). The present invention also contemplates kits wherein any one or a combination thereof of a)-j) are specifically excluded.

[0083] In a particular embodiment, which is not meant to be limiting in any manner, there is provided a kit comprising a compound as described above and a medium for growing, culturing or infecting cells with a virus and optionally, one or more cells which are capable of being infected by the virus. The kit may also comprise instructions for using any component or combination of components and/or practicing any method as described herein.

[0084] The present invention also provides a method of enhancing or increasing the infection, spread and/or titer, or cytotoxicity of a virus in cells comprising, administering the compound as described herein to the cells prior to, after or concurrently with the virus. The method is preferably practiced in vivo but in vitro applications are also contemplated.

[0085] The present invention also provides a method of enhancing or increasing the infection, spread and/or titer, or cytotoxicity of an attenuated virus or a genetically modified virus in cells comprising, administering the compound as described above to the cells prior to, after or concurrently with the attenuated or genetically modified virus.

[0086] The present invention also provides a method of enhancing or increasing the spread of an oncolytic virus in tumor or cancer cells comprising, administering the compound as described above to the cancer or tumor cells prior to, after or concurrently with the oncolytic virus. The cancer or tumor cells may be in vivo, or in vitro, preferably in vivo from a mammalian subject such as, but not limited to, a human subject.

[0087] Also provided is a method of enhancing or increasing the oncolytic activity of an oncolytic virus in cancer or tumor cells comprising, administering the compound as described above to the cancer or tumor cells prior to, concurrently with or after the oncolytic virus. The cancer or tumor cells may be in vivo, or in vitro, preferably from a mammalian subject such as, but not limited to a human subject.

[0088] The present invention also contemplates a method of producing a virus by growing the virus in an appropriate medium in the presence of the compound as described above.

[0089] The present invention also contemplates a method of producing an attenuated virus by growing the virus in an appropriate medium in the presence of the compound as described above.

[0090] The present invention also contemplates a method of producing a genetically modified virus by growing the virus in an appropriate medium in the presence of the compound as described above.

[0091] The present invention also contemplates a method of producing an oncolytic virus by growing the virus in an appropriate medium in the presence of the compound as described above.

[0092] The present invention also contemplates a method of producing a cancer vaccine by growing the virus in an appropriate medium in the presence of the compound as described above.

[0093] The present invention also contemplates a method of producing a cancer gene therapy vector by growing the virus in an appropriate medium in the presence of the compound as described above.

[0094] This summary of the invention does not necessarily describe all features of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0095] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0096] FIG. 1 shows the physicochemical properties and activity profile of parent compound VSe1 (3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one, FIG. 1a, also referred to as compound 1 herein). (A) Structure of compound 1 (3,4-dichloro-5-phenyl-5H-furan-2-one). (B) Stability of VSe1 in 20 mM phosphate buffer over time measured by HPLC. (C) VSe1 was incubated in sterile water for 0 h, 1.5 h, 3 h or 24 h before being used to treat 786-0 cells at different concentrations. 4 hours post-treatment, cells were infected with VSVΔ51 expressing firefly luciferase (VSVΔ51-FLuc at a multiplicity of infection (MOI) of 0.005. 40 hours later, virus output in viral expression units (VEUs) per milliliter was measured with a previously described luciferase reporter assay known in the art. (D) 786-0 cells were treated with VSe1 at various doses. VSe1 was removed and replaced with fresh media after 1 h, 1.5 h, 2 h, 2.5 h and 6 h. VSe1 was not removed in the control condition. 4 hours post-treatment, cells were infected with VSVΔ51 expressing firefly luciferase VSVΔ51-FLuc at an MOI of 0.005. For the condition where VSe1 was replaced with fresh media 6 h after treatment, infection was performed immediately following media replacement. 40 hours later, virus output in viral expression units (VEUs) per milliliter was measured with a previously described luciferase reporter assay known in the art;

[0097] FIG. 2 shows chemical synthesis routes for VSe1 (DCPDF) analogs starting from mucochloric acid. (a) AlCl₃ (1.5 equiv.), benzene, rt, 16 h (b) amine (3 equiv.), DMF (c) NaBH₄ (1.5 equiv.), MeOH, 0° C., 30 min. then, H₂SO₄ (1 equiv.), 20 min., 0° C. to rt. (d) H₂SO₄ cat., alcohol, rt. (e) NH₄OH, Na₂CO₃, H₂O, 0° C. to rt, 16 h (f) EtOAc, reflux, 3 hr. (g) benzylamine (1.1 equiv.), NaBH(OAc)₃ (1.5 equiv.), CHCl₃, 2 hr., rt. (h) SOCl₂, DMF, reflux, 16 hr. (i) amine (2.2 equiv.), dioxane, rt, 16 hr. (j) MeOH, H₂SO₄, reflux, 16 hr. (k) pyridine (2 equiv.), Ac₂O, rt, 6 hr. (l) CuI (0.1 equiv.), -78° C., 20 min., then RMgCl (2.5 equiv.) -78° C. to rt, 16 hr. (m) SNAC, DMSO, 40° C., 3 hr;

[0098] FIG. 3 shows the physicochemical and activity profile of pyrrole-based VSe1 derivatives (see also Table 1 for more information). (A) Stability of compound 10 in 20 mM phosphate buffer over time measured by HPLC. (B) 250 μL of a 40 mM DMSO stock solution of compound 10 was added to L-glutathione (15.4 mg, 5 mol equiv.) suspended in 250 μL of DMSO. The resulting mixture was placed in a 37° C. shaker. 10 μL aliquots were removed and quenched in 990 μL of water (containing 0.5% formic acid) at various time points, including at t=0 minutes and t=60 minutes shown above. Analysis by ESI-LC-MS allowed for the identification and quantification of compound 10 and the glutathione adduct. (C) 786-0 cells were treated with compound 10 at various doses. Compound 10 was removed and replaced with fresh media after 1 h, 1.5 h, 2 h, 2.5 h and 6 h. Compound 10 was not removed in the control condition. 4 hours post-treatment, cells were infected with VSVΔ51 expressing firefly luciferase (VSVΔ51-FLuc at a multiplicity of infection (MOI) of 0.005. For the condition where compound 10 was replaced with fresh media 6 h after treatment,

infection was performed immediately following media replacement. 40 hours later, virus output in viral expression units (VEUs) per milliliter was measured with a previously described luciferase reporter known in the art assay. (D) & (E) Pyrrole based VSe1 analogs enhance oncolytic Herpes Simplex Virus type-1 (HSV-1) replication in cancer cells. D) Mouse mammary carcinoma (4T1) cells were left untreated or, treated with VSe1 analog compound 10 for 4h at various concentrations: 2.5 μM, 5 μM, 10 μM, 15 μM or 20 μM. ICP0-null HSV-N212eGFP was then added at MOI 0.005. eGFP fluorescence was detected 48h after HSV infection (D). In (E), HSV titers were determined 48 h after infection. Mean±SEM from 3 independent experiments when error bars are shown;

[0099] FIG. 4 shows that VSe1 (or Compound 1) and its analogues selectively enhance the replication of oncolytic vesicular stomatitis virus in ex vivo tumor tissues. (A) CT26 (murine colon carcinoma) tumors were grown subcutaneously in Balb/c mice for 24 days and subsequently excised and cored, along with normal brain, lung and spleen tissues. Tissue samples were treated in triplicate with various concentrations of compounds for 4 hours prior to infection with 1×10⁴ plaque-forming units of vesicular stomatitis virus expressing GFP (VSVΔ51-GFP). Virus replication was assessed by fluorescence microscopy 24 hours post-infection. Representative images from each triplicate set for an effective concentration are shown. (B-G) Infected cores and corresponding supernatants were collected 36 hours post-infection. VSVΔ51-GFP infection was quantified by standard plaque assay (8). Cores were homogenized prior to titering. Graphs show the sum of infectious titer from core and supernatant for each compound in each tissue type. Doses shown here are those that are depicted in panel (A). The horizontal black line on each graph at 1×10⁴ PFU/mL represents the amount of VSVΔ51-GFP used to initially infect each core;

[0100] FIG. 5 shows the results of dose escalation studies with VSe1 (compound 1), compound 10, compound 24 and compound 28 in mice. Six-week old female Balb/c mice were given (A) compound 1, (B) compound 10, (C) compound 24, or (D) compound 28 dissolved in DMSO via intraperitoneal administration. Five mice were assigned to each dose group for each chemical. The dose was adjusted for individual mice based on weight. Graphs stop when the first mouse in the group was euthanized. (A-B) Mice were injected on Day 1 and weights were recorded over a 10 day period. (C-D) Mice were injected on Day 1, 3 and 5. Weights were recorded over an 18 day period. For all groups, weights are reported relative to the initial weight on Day 1;

[0101] FIG. 6 shows that pyrrole-based VSe1 analogs enhance the spread of oncolytic virus in resistant tumors in vivo. VSVΔ51-resistant CT26 cells were subcutaneously engrafted into female Balb/c mice. After 11 days (tumors were approximately 5 mm×5 mm in size), mice were given 30 μL of vehicle (DMSO) (A, C), 50 mg/kg of compound 28 (B), or 40 mg/kg of compound 10 (D) by intratumoral injection. 4 hours later, mice were treated with 1×10⁸ plaque-forming units of VSVΔ51-FLuc. Virus replication was measured by quantifying luciferase expression (in mean relative light units) with an in-vivo imaging system (IVIS) 24 hours post-infection for groups A-B and 48 hours post-infection for groups C-D;

[0102] FIG. 7 shows that VSe1 and its analogues may be combined for synergistic effects. Human renal carcinoma

(786-0) cells were treated with various concentrations of VSe1 alone, MD03011 alone, or co-treated with both compounds. After 4 hours, cells were infected with VSVΔ51 expressing firefly luciferase (VSVΔ51Fluc) at an MOI of 0.005. 40 hours later, virus output in viral expression units (VEUs) per milliliter was measured with a previously described luciferase reporter assay. Data is reported as fold change in VEU relative to the VSe1 19 μM condition (Garcia et al J Vis Exp 2014; herein incorporated by reference in its entirety);

[0103] FIG. 8 shows that VSe1 and its analogues may enhance infection of cells with oncolytic Maraba virus. Human renal carcinoma (786-0) cells were left untreated or, treated with compound at various concentrations. After 4 hours, cells were infected with MGI-eGFP at MOI 0.005. eGFP fluorescence was detected 24h after MG1 infection and quantified using a Cellomics ArrayScan high content screening microscope. Compound labels correspond to Table 1;

[0104] FIG. 9 shows that VSe1 and its analogs enhance infection of cells with Herpes Simplex Virus-1. Human renal carcinoma (786-0) cells were left untreated or, treated with compound at various concentrations. After 4 hours, cells were infected with ICP0-null HSV-N212eGFP at MOI 0.01. eGFP fluorescence was detected 48 h after HSV infection and quantified using a Cellomics ArrayScan high content screening microscope. Compound labels correspond to Table 1; and

[0105] FIG. 10 shows that VSe1 analogue 28 (see Table 1) enhances therapeutic activity of oncolytic VSV in a human colon cancer xenograft model. (A) VSVΔ51-resistant HT29 cells (human colon carcinoma) were subcutaneously engrafted into female Balb/c mice. After tumors reached a size of at least 5 mmx5 mm, mice were given 25 μL of vehicle (DMSO), or 40 mg/kg of compound 28 by intratumoral injection. Four hours later, mice were treated with 1×10^8 plaque-forming units of VSVΔ51-FLuc. Tumor volume ($\text{width}^2 \times \text{length} / 2$) was monitored using electronic calipers and average tumour volume relative to the treatment day are shown. Mice were euthanized when tumors reached a size of 1600 mm². Tumor growth curves were stopped when the first mouse in each group was euthanized. (B) Survival was monitored over time.

DETAILED DESCRIPTION

[0106] The following description is of one or more preferred embodiments. Several inventions may be described herein with compounds, compositions, and kits provided with identical, similar or distinct uses or methods of use.

[0107] In a first aspect, there is provided one or more compounds, alone or in combination which enhances or increases viral activity, for example, but not limited to, at least one of infection, production, titer, spread or cytotoxicity in cells as compared to when the one or more compounds are not employed. The compounds described herein may be considered viral sensitizing compounds, viral sensitizers or viral sensitizing agents.

[0108] In still a further embodiment, which is not meant to be limiting, there is provided one or more compounds, alone or in combination which enhances or increases at least one of oncolytic viral production or cytotoxicity in cells, for example, but not limited to, by increasing oncolytic viral titers in cells, increasing oncolytic viral spread in cells, increasing oncolytic viral cytotoxicity in cells or any com-

bination thereof, as compared to when the one or more compounds are not employed.

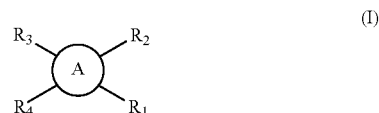
[0109] In still a further embodiment, which is not meant to be limiting, there is provided one or more compounds, alone or in combination which enhances or increases at least one of viral production or cytotoxicity in cancer or tumor cells for example, but not limited to, by increasing viral titers in cancer or tumor cells, increasing viral spread in cancer or tumor cells, increasing viral cytotoxicity in cancer or tumor cells or any combination thereof, as compared to when the one or more compounds are not employed. In a further aspect, which is not meant to be limiting, oncolytic virus production or cytotoxicity is enhanced or increased in cancer or tumor cells as compared to viral production or cytotoxicity in normal or non-cancer or tumor cells.

[0110] It will be understood by the person of skill in the art having regard to the teachings herein that enhancing or increasing viral activity, production, oncolytic activity, or cytotoxicity may include enhancing or increasing at least one of viral infection and/or rate thereof, viral production and/or rate thereof, viral titer and/or rate at which full titer may be reached, viral spread and/or rate thereof, cell lysis and/or rate thereof, viral cytotoxicity and/or rate thereof, or any combination thereof, as compared to when the one or more compounds are not used.

[0111] In still a further embodiment, which is not meant to be limiting, there is provided one or more compounds, alone or in combination which enhances or increases at least one of oncolytic viral production or cytotoxicity in cancer or tumor cells, for example, but not limited to by increasing oncolytic viral production in cancer or tumor cells, increasing oncolytic viral titers in cancer or tumor cells, increasing oncolytic viral spread in cancer or tumor cells, increasing oncolytic viral cytotoxicity in cancer or tumor cells or any combination thereof, as compared to when the one or more compounds are not employed. In a further aspect, which is not meant to be limiting, oncolytic viral production or cytotoxicity is enhanced in cancer or tumor cells as compared to oncolytic viral production or cytotoxicity in normal or non-cancer or non-tumor cells.

[0112] Based on results obtained for specific compounds in various tests and screens as described herein and having regard to the results obtained from several structure-function analyses, a broad class of compounds and several subclasses was identified which exhibit one or more of the properties as described above, or which may be employed as controls or otherwise in in-vivo or in-vitro experiments or in additional structure-function analyses to determine additional compounds with interesting features as described herein.

[0113] The present invention concerns compounds of formula



an N-oxide, pharmaceutically acceptable addition salt, quarternary amine or stereochemically isomeric form thereof, wherein:

A is a 5-membered heterocyclic ring comprising 0 or 1 double bond and 1 heteroatom selected from O, and substituted or unsubstituted N;

R₁ and R₄ are each independently H, oxo, hydroxyl, alkynyloxy, phenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, or indolyl;

R₂ and R₃ are each independently hydrogen, halogen, alkynylamino, isobutylamino, or benzylamino.

[0114] In a particular embodiment, R₄ is oxo. In a further embodiment R₁ is hydroxyl. In still a further embodiment R₄ is oxo and R₁ is hydroxyl.

[0115] In an embodiment which is not meant to be considered limiting in any manner, there is provided a viral sensitizing compound as described above, wherein A is a 5-membered heterocyclic ring comprising, for example, but not limited to, unsubstituted N or N substituted with C1-C12 alkyl, alkenyl, alkynyl, alkynyloxy, phenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, naphthalenyl, pyridinyl, furanyl, thiophenyl, sulfonobenzyl, methylsulfonobenzyl, or pyrrolyl. Other substituents are also contemplated.

[0116] In a particular embodiment, the N in the 5-membered heterocyclic ring is unsubstituted. In a further embodiment, the N in the 5-membered heterocyclic ring is substituted, for example, but without limitation with phenyl or benzyl.

[0117] In a further embodiment of the present invention, there is provided a viral sensitizing compound as described above, wherein A is a 5-membered heterocyclic ring comprising, for example, but not limited to, N substituted with methyl, ethyl, propyl, cyclopropyl, phenyl, benzyl, halogen substituted benzyl, methoxybenzyl, benzyltriazolyl, morpholinoethyl, —CH₂-C≡CH, —C≡CH, mercaptoethyl, —CH₂CH₂NH₃, CH₂-C≡C-phenyl, trifluoromethylbenzyl, or fluoroethyl.

[0118] By the term “viral sensitizing compound”, “viral sensitizing agent”, or “viral sensitizer”, it is meant a compound that increases or enhances the infection of a virus, for example, but without limitation, a genetically modified virus or attenuated virus, a cancer vaccine, a cancer gene therapy vector or more preferably an oncolytic virus; increases or enhances the spread of a virus, preferably a genetically modified virus or attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus in one or more types of cells; increases or enhances the cytotoxicity/oncolytic activity of an oncolytic virus against one or more cancer or tumor cells; increases or enhances the production, yield, titer, or reproductive capacity of a virus, more preferably a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus; or any combination of the above. It is also preferred that the viral sensitizing compound reduces the viability of a cancer or tumor cell by either killing the cancer or tumor cells or limiting its growth for a period of time.

[0119] By the term “oncolytic virus” it is meant a virus that preferentially infects and lyses cancer or tumor cells as compared to non-cancer or normal cells. In a preferred embodiment, the virus in the presence of one or more compounds or compositions described herein preferentially infects and lyses cancer cells or tumor cells as compared to the virus alone and as compared to normal cells alone or in the presence of the compound or composition alone. Cytotoxic/oncolytic activity of the virus may be present, observed or demonstrated in vitro, in vivo, or both. Prefer-

ably, the virus exhibits cytotoxic/oncolytic activity in vivo. It is to be understood that the oncolytic virus need not always preferentially infect all cancer or tumor cells over all normal cells or tissues. For example, but not wishing to be limiting, FIG. 4G shows an example of an oncolytic virus which preferentially infects and enhances replication in tumors in the presence of compound 40 even though compound 40 also increases infection and replication in brain tissue in the presence of compound 40.

[0120] Additional examples of oncolytic viruses known in the art which may be employed herein include, without limitation, reovirus, newcastle disease virus, adenovirus, herpes virus, polio virus, mumps virus, measles virus, influenza virus, vaccinia virus, rhabdoviruses such as vesicular stomatitis virus and derivatives/variants thereof.

[0121] By a “derivative” or “variant” of a virus, it is meant a virus obtained by selecting the virus under different growth conditions, one that has been subjected to a range of selection pressures, that has been genetically modified using recombinant techniques known within the art, or one that has been engineered to be replication defective and/or express transgenes, or any combination thereof. Examples of such viruses are known in the art, for example from US patent applications 20040115170, 20040170607, 20020037543, WO 00/62735; U.S. Pat. Nos. 7,052,832, 7,063,835, 7,122, 182 (which are hereby incorporated by reference) and others. Preferably the virus is a Vesicular stomatitis virus (VSV), or a related rhabdovirus variant/derivative thereof, for example, selected under specific growth conditions, one that has been subjected to a range of selection pressures, one that has been genetically modified using recombinant techniques known within the art, or a combination thereof. In a preferred embodiment, the virus is VSVΔ51 (Stojdl et al., VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents., *Cancer Cell*. 2003 October; 4(4):263-75, herein incorporated by reference). Other derivatives or variants may be based on viruses such as Maraba MG-1, Rabies, Rotavirus, Influenza, Hepatitis A, Mumps, Measles, Rubella, Herpesvirus, Reovirus, Parvovirus, Dengue Virus, Cickungunya Virus, Vaccinia virus, Modified Vaccinia Ankara, Respiratory Syncytial Virus, Varicella, LCMV, HSV-1, HSV-2, adenovirus, adeno-associated virus, lentivirus, or replicating retrovirus.

[0122] The one or more types of cancer or tumor cells may be cancer or tumor cells in vitro or in vivo from any cell, cell line, tissue or organism, for example, but not limited to human, rat, mouse, cat, dog, pig, primate, horse and the like. In a preferred embodiment, the one or more cancer or tumor cells comprise human cancer or tumor cells, for example, but not limited to lymphoblastic leukemia, myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytoma, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, malignant fibrous histiocytoma, brain stem glioma, brain tumor, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, craniopharyngioma, ependymoblastoma, medulloblastoma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma, visual pathway and hypothalamic glioma, spinal cord tumors, breast cancer, bronchial tumors, Burkitt lymphoma, carcinoid tumor, central nervous system lymphoma, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myelogenous leuke-

mia, chronic myeloproliferative disorders, colon cancer, cutaneous T-Cell lymphoma, embryonal tumors, endometrial cancer, ependymoblastoma, ependymoma, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), gastrointestinal stromal cell tumor, germ cell tumors, extracranial, extragonadal, ovarian, gestational trophoblastic tumor, glioma, hairy cell leukemia, head and neck cancer, hepatocellular (Liver) cancer, histiocytosis, Langerhans cell cancer, Hodgkin lymphoma, hypopharyngeal cancer, islet cell tumors, Kaposi sarcoma, kidney cancer, laryngeal cancer, lymphocytic leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, non-small cell lung cancer, small cell lung cancer, Hodgkin lymphoma, non-Hodgkin lymphoma, malignant fibrous histiocytoma of bone and osteosarcoma, medulloblastoma, medulloepithelioma, melanoma, intraocular melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal parenchymal tumors, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter cancer, transitional cell cancer, respiratory tract carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, uterine sarcoma, skin cancer, Merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (Gastric) cancer, supratentorial primitive neuroectodermal tumors, T-Cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, trophoblastic tumor, urethral cancer, uterine cancer, endometrial cancer, uterine sarcoma, vaginal cancer, vulvar cancer, or Wilms tumor. However, the compounds and compositions described herein possible may be used to treat other cancers or tumor in vivo or in vitro.

[0123] The present invention also provides a composition comprising a) one or more compounds as described herein and b) one or more additional components, for example, but not limited to 1) a carrier, diluent or excipient, 2) a pharmaceutically acceptable carrier, diluent or excipient, 3) a virus, for example, but not limited to an attenuated virus, a genetically modified virus or an oncolytic virus, 4) cancer or tumor cells, 5) non-cancerous or normal cells, 6) cell culture media, 7) one or more cancer therapeutics, for example, but not limited to chemotherapeutics. As an example, but not to be considered limiting in any manner, cyclophosphamide (CPA) is a common chemotherapy drug used primarily for the treatment of lymphoma, chronic lymphocytic leukemia and breast, ovarian and bladder cancers. CPA is converted into its active metabolites, 4-hydroxycyclophosphamide and aldophosphamide by liver oxidases. Use of CPA as an immune suppressant to enhance viral oncolysis has improved virotherapy efficacy in combination with oncolytic variants of HSV, adenoviruses, measles virus, reovirus, and vaccinia virus.

[0124] A further cancer therapeutic known in the art is cisplatin. Cisplatin binds and cross-links cellular DNA leading to apoptosis when DNA is not repaired. Cisplatin has been investigated in combination with oncolytic adenoviruses, herpes viruses, parvovirus, vaccinia virus, and vesicular stomatitis virus. Enhanced therapeutic activity in vitro and in vivo has been observed when combining cisplatin with oncolytic variants of adenovirus, herpesvirus, parvovirus and vaccinia virus whereas slight inhibition was observed for oncolytic variant of vesicular stomatitis virus.

[0125] Mitomycin C (MMC) is a DNA cross-linking antibiotic with antineoplastic properties. MMC exhibited synergistic cytotoxicity with oncolytic HSV. In vivo, combination of oncolytic herpes virus and MMC significantly improved therapeutic effects in models of gastric carcinomatosis and non-small cell lung cancer.

[0126] Doxorubicin is an anthracycline antibiotic that intercalates into DNA and prevents the action of topoisomerase II. Doxorubicin was synergistically cytotoxic when combined with oncolytic adenovirus (ONYX-015) and the combination reduced tumor growth relative to the monotherapies. ONYX-015 was successfully combined with MAP (mitomycin C, doxorubicin and cisplatin) chemotherapy in a phase I-II clinical trial for treatment of advanced sarcomas.

[0127] Gancyclovir (GCV) is a widely used antiviral agent, originally developed for the treatment of cytomegalovirus infections. GCV is a guanosine analogue prodrug that upon phosphorylation by herpes virus thymidine kinase (TK) competes with cellular dGTP for incorporation into DNA resulting in elongation termination. Oncolytic viruses encoding the HSV TK gene lead to an accumulation of toxic GCV metabolites in tumor cells which interfere with cellular DNA synthesis leading to apoptosis. Targeted oncolytic HSV viruses in combination with GCV significantly improved survival in models of human ovarian cancer and rat gliosarcoma. Adenoviruses, engineered to express the HSV TK gene, also show enhanced anti-tumor activity when combined with GCV.

[0128] CD/5-FC enzyme/pro-drug therapy has also proven successful in combination with oncolytic virotherapy. 5-FU is a pyrimidine analogue that inhibits the synthesis of thymidine. The anti-tumor activity of two different oncolytic vaccinia viruses expressing CD was significantly enhanced when combined with 5-FC therapy in immune-competent ovarian cancer and immune suppressed colon cancer models.

[0129] Taxanes are a class of chemotherapy drugs, including paclitaxel and docetaxel, which cause stabilization of cellular microtubules thereby preventing function of the cellular cytoskeleton, a requirement for mitosis. Combination of docetaxel or paclitaxel with an urothelium- or prostate-targeted oncolytic adenovirus significantly reduced in vivo tumor volume and resulted in synergistic in vitro cytotoxicity.

[0130] Rapamycin (sirolimus) is an immunosuppressant commonly used in transplant patients however it has also been shown to significantly enhance the oncolytic effects of the oncolytic variants of poxviruses myxoma and vaccinia virus.

[0131] The prototypical proteasome inhibitor MG-132 enhanced cellular CAR expression in Lovo colon carcinoma cells, which was accompanied with enhanced oncolytic adenovirus target gene expression and oncolysis.

[0132] The efficacy of oncolytic VSV against chronic lymphocytic leukemia cells was increased by combination therapy with the BCL-2 inhibitor EM20-25.

[0133] One group showed that a single dose of angiostatic cRGD peptide treatment before oncolytic virus treatment enhanced the antitumor efficacy of oncolytic HSV.

[0134] The present invention also provides a kit comprising one or more compound(s) alone or in combination or a composition comprising same, for example as described herein. The kit may also comprise one or more of a cell culture dish/plate or multi-well dish/plate, and/or an apparatus or device(s) to deliver the compound(s) or a composition comprising the same to a cell, tissue, cell culture or cell culture medium, or to a subject in vivo. The kit may also comprise instructions for administering or using the compound(s), and/or virus(es) as described herein, for example, but not limited to attenuated viruses, genetically modified viruses, cancer vaccines, cancer gene therapy vectors, oncolytic viruses, any combination thereof, or any combination of distinct viruses.

[0135] For in vivo therapeutic applications, preferably there is provided a pharmaceutical composition comprising one or more compounds and a pharmaceutically acceptable carrier, diluent or excipient, optionally containing other solutes such as dissolved salts and the like. In a preferred embodiment, the solution comprises enough saline, glucose or the like to make the solution isotonic.

[0136] Pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "Remington: The Science and Practice of Pharmacy" (formerly "Remington's Pharmaceutical Sciences"); Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, Pa. (2000), herein incorporated by reference.

[0137] Administration of such compositions may be via a number of routes depending upon whether local and/or systemic treatment is desired and upon the area to be treated. In a first embodiment, which is not meant to be limiting, the compound is administered locally to the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary (e.g. by inhalation or insufflation of powders or aerosols, including by nebulizer), intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, or intracranial, e.g. intrathecal or intraventricular, administration. Also contemplated is intra-tumor injection, perfusion or delivery into the general vicinity of the tumor or injection into the vasculature supplying a tumor. Alternatively, the viral sensitizing compounds may be formulated in a tablet or capsule for oral administration. Alternate dosage forms, including slow-release, sustained-release, extended release, as would be known in the art are also contemplated.

[0138] For administration by inhalation or insufflation, the compounds can be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. For topical use, the modulators can be formulated as dusting powders, creams or lotions in pharmaceutically acceptable vehicles, which are applied to affected portions of the skin.

[0139] Without wishing to be limiting, the dosage requirements for the viral sensitizing compounds of the present invention may vary with the particular compositions

employed, the route of administration and the particular subject being treated. Dosage requirements can be determined by standard clinical techniques known to a worker skilled in the art. Typically, treatment will generally be initiated with small dosages less than the optimum dose of the compound or compound/virus. Thereafter, the dosage is increased until the optimum or satisfactory effect under the circumstances is reached. In general, the viral sensitizing agent or pharmaceutical compositions comprising the viral sensitizing agent are administered at a concentration that will generally afford effective results without causing significant harmful or deleterious side effects. Administration can be either as a single unit dose or, if desired, the dosage can be divided into convenient subunits that are administered at suitable times throughout the day.

[0140] The viral sensitizing compound may be employed in sequential administration, for example, before, after or both before and after administration of a virus, for example, but not limited to an attenuated virus, a genetically modified virus, a cancer vaccine, a cancer gene therapy vector or an oncolytic virus. Alternatively, the viral sensitizing compound may be administered concurrently or in combination with a virus as described above, preferably in combination with an oncolytic virus. In addition, the viral sensitizing agent may be used with an oncolytic virus as described above and in combination with one or more cancer therapeutics or cancer therapies as is known to a person of skill in the art, for example but not limited to interferon therapy, interleukin therapy, colony stimulating factor therapy, chemotherapeutic drugs, for example, but not limited to 5-fluorodeoxyuridine, amsacrine, bleomycin, busulfan, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clofarabine, crisantaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil, gemcitabine, gliadel, hydroxycarbamide, idarubicin, ifosfamide, irinotecan, leucovorin, lomustine, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, pentostatin, procarbazine, raltitrexed, satraplatin, streptozocin, tegafur-uracil, temozolomide, teniposide, thiopeta, tioguanine, topotecan, treosulfan, vinblastine, vincristine, vindesine, vinorelbine or a combination thereof. Further, anti-cancer biologics may also be employed, for example, but without limitation, monoclonal antibodies and the like.

[0141] The present invention also contemplates methods and uses of the compounds as described herein for increasing or enhancing the spread of a virus, for example, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus in one or more cells, for example, but not limited to one or more types of cancer or tumor cells, increasing or enhancing the cytotoxicity/oncolytic activity of an oncolytic virus against one or more cancer or tumor cells, increasing or enhancing the production, yield or reproductive capacity of a virus, for example, a genetically modified virus, an attenuated virus, cancer vaccine, cancer gene therapy vector, an oncolytic virus, or, any combination of the above. In an embodiment, which is not meant to be limiting in any manner, the viral sensitizing compound reduces the viability of a cancer or tumor cell by either killing the cancer or tumor cell or limiting its growth for a period of time. The compounds may also be used for the production of a medicament for accomplishing same.

[0142] In an embodiment of the present invention, which is not meant to be limiting in any manner, the one or more compounds are α,β -dichloro- γ -hydroxy-N-benzyl-crotonic lactam; 3,4-dichloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dibromo-5-prop-2-ynyloxy-5H-furan-2-one; 3-chloro-5-phenyl-4-prop-2-ynylamino-5H-furan-2-one; 3-chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one; 1-benzyl-3,4-dichloro-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-(2-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one; 4-benzylamino-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-prop-2-ynyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5H-furan-2-one; benzo[1,3]dioxole-5,6-dione; 4,5-dichloro-2H-pyridazin-3-one; 4,5-dichloro-2-phenyl-2H-pyridazin-3-one; 3,4-dichloro-1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-5-(1H-indol-3-yl)-5H-furan-2-one; indole-3-crotonic acid; 3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 3,4-dichloro-5-(3-nitro-phenyl)-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-prop-2-ynyl-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-(2-chloro-benzyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-propyl-1,5-dihydro-pyrrol-2-one; 1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-1-propyl-pyrrole-2,5-dione; 1-benzyl-3,4-dichloro-pyrrole-2,5-dione; 3,4-dichloro-5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one; 1-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; [4-(4-chloro-3-isobutylamino-5-oxo-2,5-dihydro-furan-2-ylloxymethyl)-[1,2,3]triazol-1-yl]-acetic acid; [4-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-pyrrol-1-ylmethyl)-[1,2,3]triazol-1-yl]-acetic acid; 3,4-dichloro-5-hydroxy-1-phenethyl-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-morpholinoethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-cyclopropyl-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-mercaptoethyl)-1H-pyrrol-2(5H)-one; 2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethanaminium 2,2,2-trifluoroacetate; 3,4-dichloro-5-hydroxy-1-(3-phenylprop-2-ynyl)-1H-pyrrol-2

(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(trifluoromethyl)benzyl)-1H-pyrrol-2(5H)-one; 1-(biphenyl-4-ylmethyl)-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-nitrobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-methoxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(2-chlorobenzyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 1-benzhydryl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(naphthalen-1-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(1-phenylethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-3-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-4-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(furan-2-ylmethyl)-5-hydroxy-1H-pyrrol-2(5H)-one; N-(2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide; (3aS)-2,3-dichloro-5-phenyl-4,5-dihydropyrrolo[1,2-a]quinolin-1 (3aH)-one; 3,4-diiodo-2-phenyl-2,5-dihydrofuran; D-Gluconamide, N-octyl; (S)-11-amino-4,7,10,14-tetraoxo-15-((2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-3,6,9,13-tetraazapentadecan-1-oic acid; 1-allyl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-hydroxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(thiophen-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(methylsulfonyl)benzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-((4,5-dimethyloxazol-2-yl)methyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(3,4,5-trifluorobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one; 4,5-dichloro-2-(2,2,2-trifluoroethyl)pyridazin-3(2H)-one; 4,5-dichloro-2-cyclohexylpyridazin-3 (2H)-one; methyl 2-(4-((3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate; 4,5-dichloro-2-otolylpyridazin-3(2H)-one; 4,5-dichloro-2-(2-(dimethylamino)ethyl)pyridazin-3(2H)-one hydrochloride; and 4,5-dichloro-2-(4-fluorophenyl)pyridazin-3(2H)-one or any compound or group of compounds as described herein. Table 2 below includes structures of several interesting viral sensitizing compounds such as these.

TABLE 2

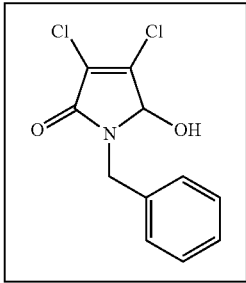
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01145	α,β -dichloro- γ -hydroxy-N-benzyl-crotonic lactam	

TABLE 2-continued

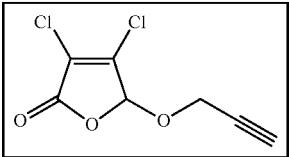
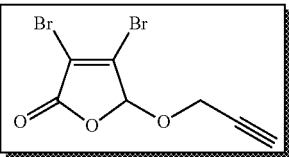
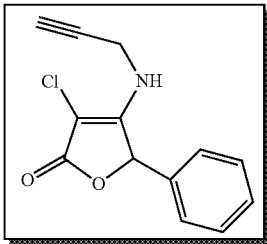
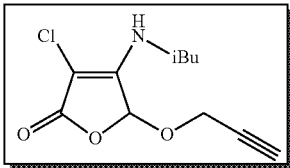
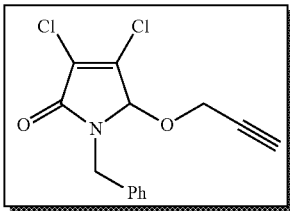
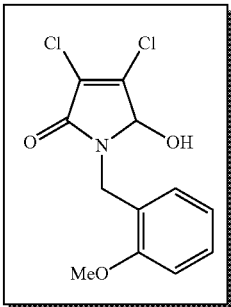
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01155, MD02140	3,4-Dichloro-5-prop-2-ynyloxy-5H-furan-2-one,	
CM01013, MD02182	3,4-Dibromo-5-prop-2-ynyloxy-5H-furan-2-one	
CM01027	3-Chloro-5-phenyl-4-prop-2-ynylamino-5H-furan-2-one	
MD01165, MD02142	3-Chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one	
MD01171, MD02180	1-Benzyl-3,4-dichloro-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one	
CM01025	3,4-Dichloro-5-hydroxy-1-(2-methoxybenzyl)-1,5-dihydro-pyrrol-2-one	

TABLE 2-continued

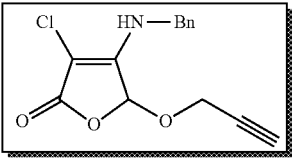
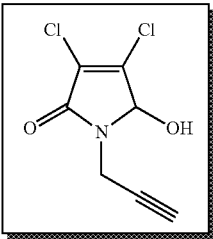
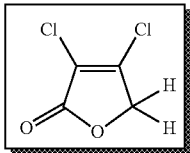
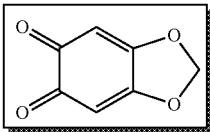
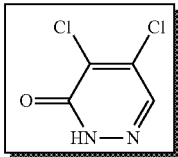
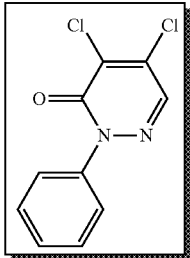
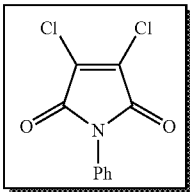
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01159	4-Benzylamino-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one	
MD01151	3,4-Dichloro-5-hydroxy-1-prop-2-ynyl-1,5-dihydro-pyrrol-2-one	
MD02068	3,4-Dichloro-5H-furan-2-one	
TD193	Benzo[1,3]dioxole-5,6-dione	
MD02026	4,5-Dichloro-2H-pyridazin-3-one	
MD02054	4,5-Dichloro-2-phenyl-2H-pyridazin-3-one	
MD01139	3,4-Dichloro-1-phenyl-pyrrole-2,5-dione	

TABLE 2-continued

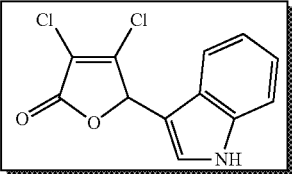
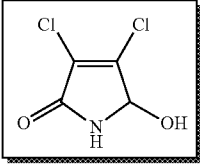
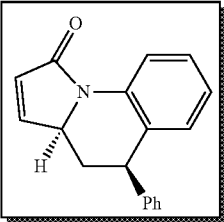
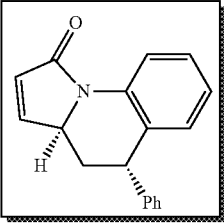
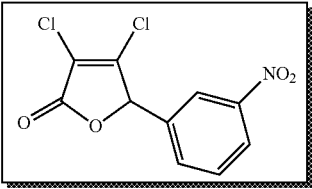
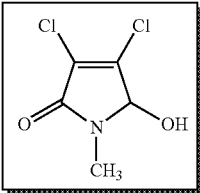
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01041	3,4-Dichloro-5-(1H-indol-3-yl)-5H-furan-2-one, indole-3-crotonic acid	
MD01033, MD02052	3,4-Dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one	
MD01071F1	5-Phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one	
MD01071F2	5-Phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one	
MD01085	3,4-Dichloro-5-(3-nitro-phenyl)-5H-furan-2-one	
CM01031, CP01026	3,4-Dichloro-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one	

TABLE 2-continued

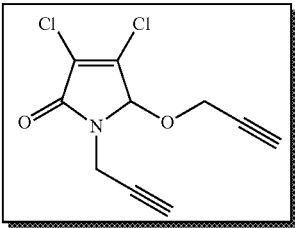
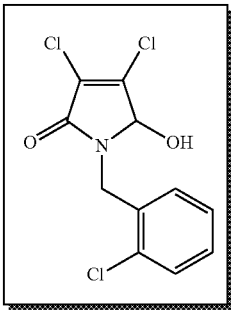
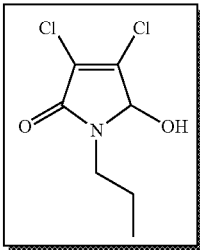
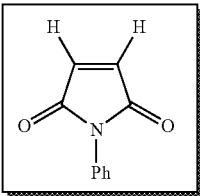
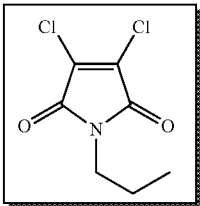
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01169	3,4-Dichloro-1-prop-2-ynyl-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one	
MD01179	3,4-Dichloro-1-(2-chloro-benzyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one	
MD02010	3,4-Dichloro-5-hydroxy-1-propyl-1,5-dihydro-pyrrol-2-one	
MD01037	1-Phenyl-pyrrole-2,5-dione	
MD01129	3,4-Dichloro-1-propyl-pyrrole-2,5-dione	

TABLE 2-continued

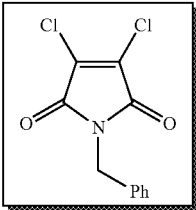
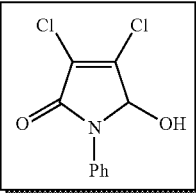
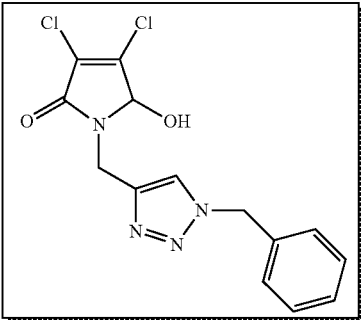
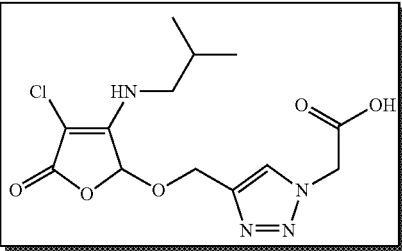
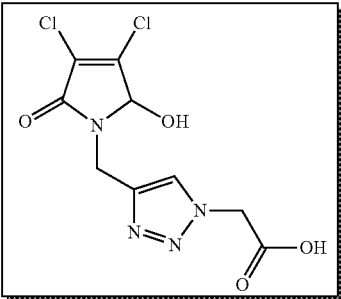
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01133	1-Benzyl-3,4-dichloro-pyrrole-2,5-dione	
MD01147	3,4-Dichloro-5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one	
CM01099	1-(1-Benzyl-1H-[1,2,3]triazol-4-ylmethyl)-3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one	
MD02136	[4-(4-Chloro-3-isobutylamino-5-oxo-2,5-dihydro-furan-2-ylloxymethyl)-[1,2,3]triazol-1-yl]-acetic acid	
MD02124	[4-(3,4-Dichloro-2-hydroxy-5-oxo-2,5-dihydro-pyrrol-1-ylmethyl)-[1,2,3]triazol-1-yl]-acetic acid	

TABLE 2-continued

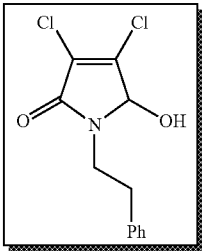
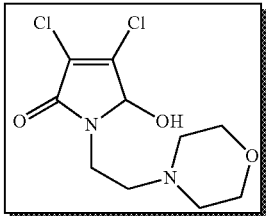
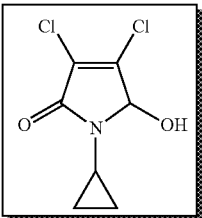
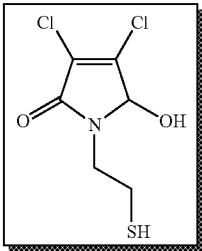
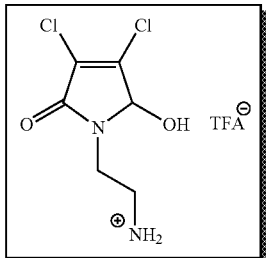
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD03009	3,4-dichloro-5-hydroxy-1-phenethyl-1H-pyrrol-2(5H)-one	
MD03011	3,4-dichloro-5-hydroxy-1-(2-morpholinoethyl)-1H-pyrrol-2(5H)-one	
MD03013	3,4-dichloro-1-cyclopropyl-5-hydroxy-1H-pyrrol-2(5H)-one	
MD03007	3,4-dichloro-5-hydroxy-1-(2-mercaptoethyl)-1H-pyrrol-2(5H)-one	
CP01046	2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethanaminium 2,2,2-trifluoroacetate	

TABLE 2-continued

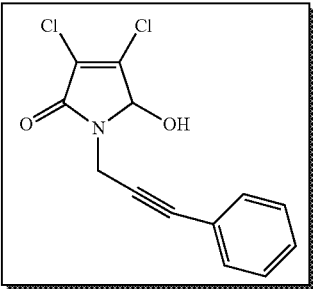
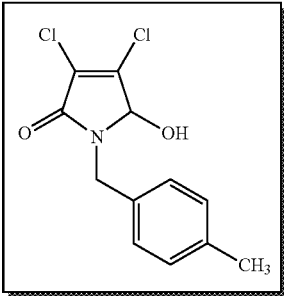
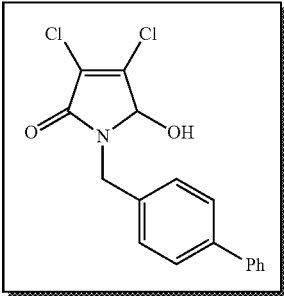
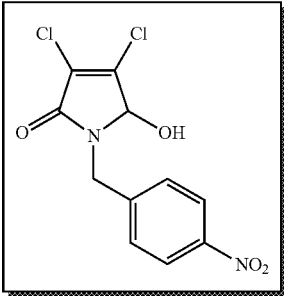
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01183	3,4-dichloro-5-hydroxy-1-(3-phenylprop-2-ynyl)-1H-pyrrol-2(5H)-one	
MD03017	3,4-dichloro-5-hydroxy-1-(4-(trifluoromethyl)benzyl)-1H-pyrrol-2(5H)-one	
CP01042	1-(biphenyl-4-ylmethyl)-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one	
CP01001	3,4-dichloro-5-hydroxy-1-(4-nitrobenzyl)-1H-pyrrol-2(5H)-one	

TABLE 2-continued

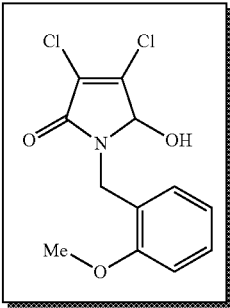
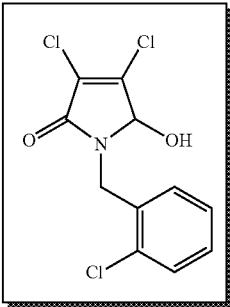
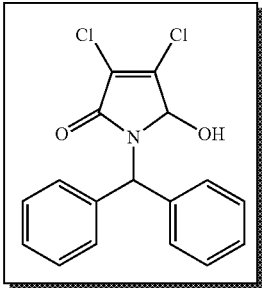
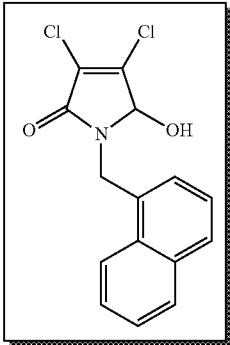
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
CP01005	3,4-dichloro-5-hydroxy-1-(2-methoxybenzyl)-1H-pyrrol-2(5H)-one	
CP01011	3,4-dichloro-1-(2-chlorobenzyl)-5-hydroxy-1H-pyrrol-2(5H)-one	
CP01035	1-benzhydryl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one	
CP01039	3,4-dichloro-5-hydroxy-1-(naphthalen-1-ylmethyl)-1H-pyrrol-2(5H)-one	

TABLE 2-continued

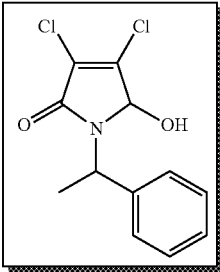
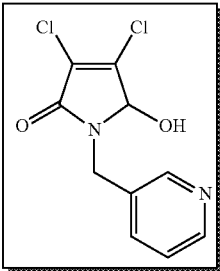
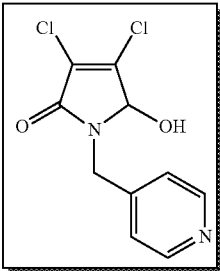
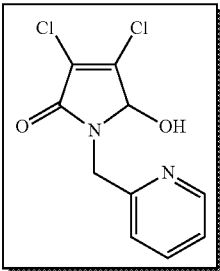
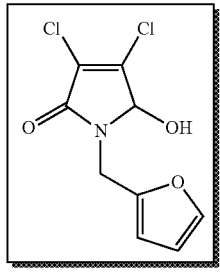
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
CP01037	3,4-dichloro-5-hydroxy-1-(1-phenylethyl)-1H-pyrrol-2(5H)-one	
CP01047	3,4-dichloro-5-hydroxy-1-(pyridin-3-ylmethyl)-1H-pyrrol-2(5H)-one	
CP01048	3,4-dichloro-5-hydroxy-1-(pyridin-4-ylmethyl)-1H-pyrrol-2(5H)-one	
CP01036	3,4-dichloro-5-hydroxy-1-(pyridin-2-ylmethyl)-1H-pyrrol-2(5H)-one	
PL01010	3,4-dichloro-1-(furan-2-ylmethyl)-5-hydroxy-1H-pyrrol-2(5H)-one	

TABLE 2-continued

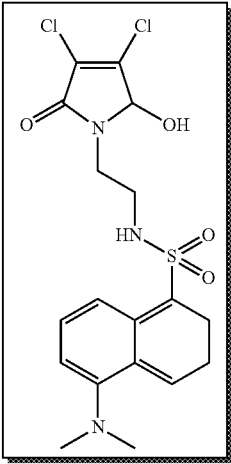
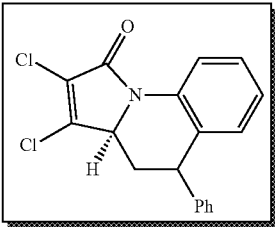
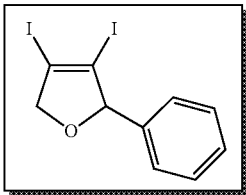
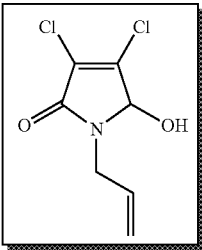
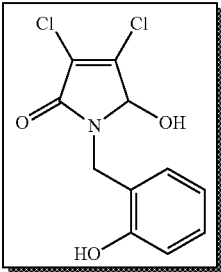
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
CP01020	N-(2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide	
CP01034	(3aS)-2,3-dichloro-5-phenyl-4,5-dihydropyrrolo[1,2-a]quinolin-1(3aH)-one	
CP01012	3,4-diiodo-2-phenyl-2,5-dihydrofuran	
PL01013	1-allyl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one	
PL01017	3,4-dichloro-5-hydroxy-1-(2-hydroxybenzyl)-1H-pyrrol-2(5H)-one	

TABLE 2-continued

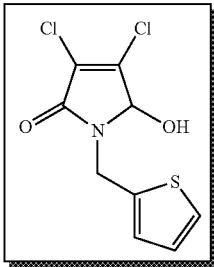
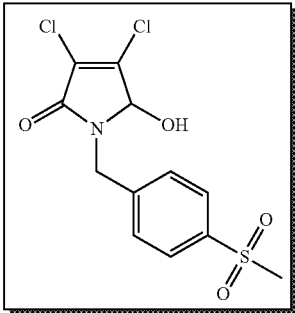
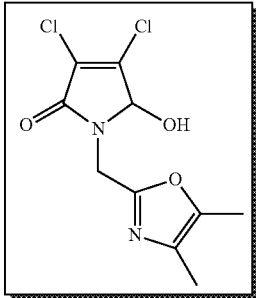
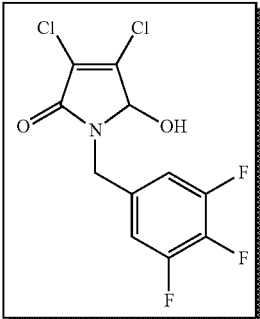
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
PL01018	3,4-dichloro-5-hydroxy-1-(thiophen-2-ylmethyl)-1H-pyrrol-2(5H)-one	
PL01019	3,4-dichloro-5-hydroxy-1-(4-(methylsulfonyl)benzyl)-1H-pyrrol-2(5H)-one	
PL01020	3,4-dichloro-1-((4,5-dimethyloxazol-2-yl)methyl)-5-hydroxy-1H-pyrrol-2(5H)-one	
PL01021	3,4-dichloro-5-hydroxy-1-(3,4,5-trifluorobenzyl)-1H-pyrrol-2(5H)-one	

TABLE 2-continued

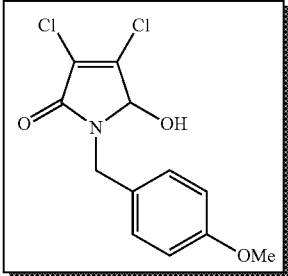
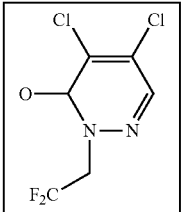
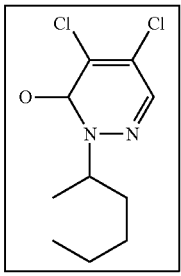
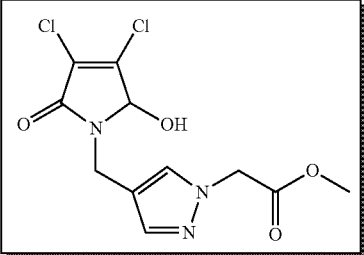
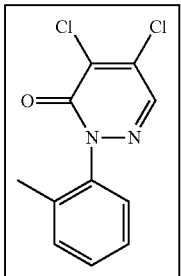
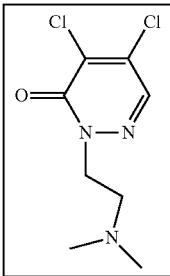
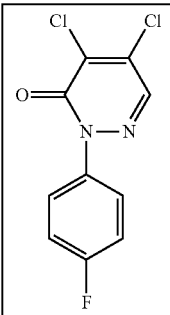
Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
MD01187	3,4-dichloro-5-hydroxy-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one	
PL01023	4,5-dichloro-2-(2,2,2-trifluoroethyl)pyridazin-3(2H)-one	
PL01024	4,5-dichloro-2-cyclohexylpyridazin-3(2H)-one	
PL01012	methyl 2-(4-((3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate	
PL01025	4,5-dichloro-2-o-tolylpyridazin-3(2H)-one	

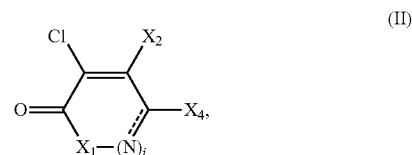
TABLE 2-continued

Structures, Chemical Names and Reference Codes for Viral Sensitizing Compounds		
Name	Chemical name	Structure
PL01026	4,5-dichloro-2-(2-(dimethylamino)ethyl)pyridazin-3(2H)-one hydrochloride	
PL01027	4,5-dichloro-2-(4-fluorophenyl)pyridazin-3(2H)-one	

[0143] As will be appreciated by a person of skill in the art, the general class structures and specific compounds as identified herein may be employed alone or in combination in any variety of compositions as required by a person of skill in the art. Without wishing to be bound by theory, potential uses for the compounds as described herein may include increasing infection, spread and/or viral titer in specific cells, for example, in cancer or tumor cells/tissues or cells derived from cultures that have been immortalized, increasing cytotoxicity of viruses, including oncolytic viruses in specific cells, for example, in cancer or tumor cells/tissues, for the production of viruses which may be subsequently used in the production of cancer gene therapy vectors or cancer vaccines. Also, importantly, the compounds as described herein may also be employed as internal controls or in structure-function analyses to determine additional classes or specific molecules which exhibit similar or improved properties to those currently described herein.

[0144] As Vse1 (compound 1) is known in the art, it is contemplated that any of the compositions described herein, uses thereof which employ this compound may be specifically excluded if desired. Any other compound or use thereof as described herein also may be specifically excluded if that compound and/or the use of it as described herein is disclosed in the art.

[0145] In an embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (II):



or

[0146] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0147] X_1 is a heteroatom such as O, NH, or substituted N;

[0148] X_2 is halogen (such as, for example, Cl), or NHX_3 , wherein X_3 is a substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl;

[0149] i is 0 when X_1 is O, or 0 or 1 when X_1 is NH or substituted N;

[0150] --- represents a double bond which is present when i is 1, and absent when i is 0 such that X_1 is directly bonded to the X_4 -bearing carbon through a single bond when i is 0; and

[0151] X_4 is H, OH, =O, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl (such as, for example, substituted or unsubstituted phenyl), or OX_{10} , wherein X_{10} is H, linear or branched substituted or unsubstituted alkyl, alkenyl, alkynyl, or acyl (for example, X_{10} may be acetyl, methyl, or $-CH_2-C\equiv CH$);

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0152] In certain non-limiting embodiments, substituted N may include N substituted with H, substituted or unsubstituted linear or branched C_1 - C_{12} alkyl, alkenyl, or alkynyl, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl, substituted or unsubstituted cycloalkyl or heterocycloalkyl, for example. For example, N may be substituted with substituted or unsubstituted alkynyloxy, phenyl, alkylphenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, naphthalenyl, substituted naphthalenyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted furanyl or thiofuranyl, thiophenyl, sulfonobenzyl, methylsulfonobenzyl, pyrrolyl, substituted or unsubstituted morpholine, cycloalkyl, alkylthiol, substituted or unsubstituted alkyamine, or substituted or unsubstituted oxazoline.

[0153] In certain non-limiting embodiments, substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl may include any suitable substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, such as an optionally substituted linear or branched alkyl, alkenyl, or alkynyl comprising a C_1 - C_{12} carbon chain and, in the case of alkenyl or alkynyl, at least one carbon-carbon double or triple bond, respectively.

[0154] In certain non-limiting embodiments, substituted or unsubstituted aryl or heteroaryl may include any suitable mono- or bi-cyclic aryl or heteroaryl group which may be optionally substituted. Examples of aryl and heteroaryl groups may include 5-membered, 6-membered, or >6-membered aryl or heteroaryl groups.

[0155] In certain non-limiting embodiments, acyl may include a group having the formula $R-C(=O)-$, wherein R is substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, for example.

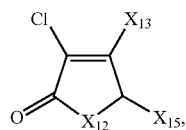
[0156] In certain non-limiting embodiments, substituted or unsubstituted cycloalkyl or heterocycloalkyl may include any suitable cycloalkyl or heterocycloalkyl group having a ring size which is ≥ 3 , and which may be optionally substituted.

[0157] In certain non-limiting embodiments, substituted or unsubstituted alkynyloxy may include any suitable group having the formula $-O-R$, wherein R comprises a substituted or unsubstituted linear or branched C_1 - C_{12} alkynyl group (i.e. a carbon chain having at least one carbon-carbon triple bond).

[0158] Non-limiting examples of suitable substituents of compounds of formula (II) may be found in the compound structures shown in Tables 1 and 2, for example.

[0159] Examples of compounds of formula (II) are described in detail herein, and may be found in both Tables 1 and 2. Subsets of compounds of formula (II), which share certain structural and/or pharmacophore features therewith, may include:

[0160] Compounds defined by formula (VIII):



(VIII)

or

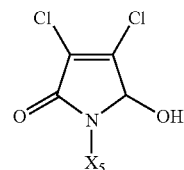
[0161] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0162] X_{12} is O, NH, or substituted N;

[0163] X_{13} is halogen such as Cl, or NHX_{14} , wherein X_{14} is a substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl; and

[0164] X_{15} is H, OH, $=O$, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl such as substituted or unsubstituted phenyl, or OX_{16} , wherein X_{16} is H, linear or branched substituted or unsubstituted alkyl, alkenyl, alkynyl, or acyl, or X_{16} is acetyl, methyl, or $-CH_2-C\equiv CH$;

[0165] Compounds defined by formula (III):



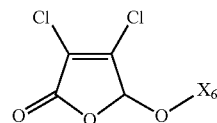
(III)

or

[0166] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0167] X_5 is H, substituted or unsubstituted linear or branched C_1 - C_{12} alkyl, alkenyl, or alkynyl, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl, substituted or unsubstituted cycloalkyl or heterocycloalkyl, or wherein X_5 is substituted or unsubstituted alkynyloxy, phenyl, alkylphenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, naphthalenyl, substituted naphthalenyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted furanyl or thiofuranyl, thiophenyl, sulfonobenzyl, methylsulfonobenzyl, pyrrolyl, substituted or unsubstituted morpholine, cycloalkyl, alkylthiol, substituted or unsubstituted alkyamine, or substituted or unsubstituted oxazoline;

[0168] Compounds defined by formula (IV):



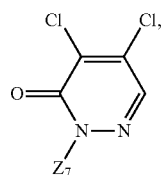
(IV)

or

a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0169] wherein X_6 is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or wherein X_6 is substituted or unsubstituted methyl, alkyl triazolyl, acetyl, or $-CH_2-C\equiv CH$;

[0170] Compounds defined by formula (V):



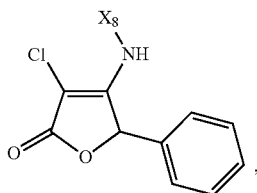
(V)

or

[0171] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0172] X_7 is H, substituted or unsubstituted aryl or heteroaryl, substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted cycloalkyl. For example, X_7 may be substituted or unsubstituted alkylamine, or substituted or unsubstituted phenyl;

[0173] Compounds defined by formula (VI):



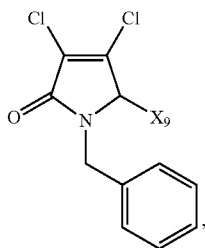
(VI)

or

[0174] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0175] wherein X_8 is substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl, or X_8 is substituted or unsubstituted benzyl; and/or

[0176] Compounds defined by formula (VII):



(VII)

or

[0177] a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

[0178] X_9 is H, OH, OX_{11} , or $=O$, wherein X_{11} is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or X_{11} is acetyl, methyl, or $-CH_2-C\equiv CH$.

[0179] In an embodiment of any one of the method or methods described herein, the compound or combination of

compounds described above may be present in a composition comprising the compound(s) and a carrier, diluent or excipient.

[0180] In another embodiment, there is provided herein a composition comprising one or more of the compounds having a structure as defined above, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more cancer cells, c) a carrier, diluent or excipient, d) a pharmaceutically acceptable carrier, diluent or excipient, e) non-cancer cells; f) cell culture media; g) one or more cancer therapeutics; or any combination of a)-g).

[0181] It will be understood by the person of skill in the art having regard to the teachings herein that two or more of the compounds described herein may be used in combination. For example, two or more of the compounds described herein may be administered to a cell simultaneously, sequentially, or in combination. As shown in FIG. 7 and described in further detail in the examples below, using VSe1 and MD03011 in combination produced a remarkable synergistic effect under the conditions tested, demonstrating that combinations of two or more compounds as described herein may be desirable in certain applications.

[0182] In yet another embodiment, there is provided herein a kit comprising one or more of the compounds having a structure as defined above, and one or more of a) a virus, a genetically modified virus, an attenuated virus, a cancer vaccine, a cancer gene therapy vector, or an oncolytic virus, b) one or more cancer cells, c) a pharmaceutically acceptable carrier, diluent or excipient, d) non-cancer cells; e) cell culture media; f) one or more cancer therapeutics, g) a cell culture plate or multi-well dish; h) an apparatus to deliver the compound to a cell, medium or to a subject; i) instructions for using the compound or any component in the kit, j) a carrier diluent or excipient, or any combination of a)-j).

[0183] In another embodiment, the cells may be cancer cells in vivo, or in vitro. In a further embodiment, the in vivo cancer cells may be from a mammalian subject. In still a further embodiment, the mammalian subject may be a human subject.

[0184] In yet another embodiment, there is provided herein a method of increasing the oncolytic activity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, having a structure as described above to said cancer or tumor cells prior to, concurrently with or after the oncolytic virus. In another embodiment, the cancer cells may be in vivo, or in vitro. In still another embodiment, the in vivo cancer cells may be from a mammalian subject. In yet another embodiment, the mammalian subject may be a human subject.

[0185] In another embodiment, there is provided herein a compound having a structure as described above. In still another embodiment, the compound may be for use as a viral sensitizer for sensitizing a cancer or tumor cell to an oncolytic virus.

[0186] In an embodiment, there is provided herein a compound having a structure as described above, for use as a viral sensitizer to enhance or increase oncolytic virus activity in cancer or tumor cells. In a further embodiment, the compound may be for enhancing or increasing oncolytic virus titer in cancer or tumor cells. In another embodiment, the compound may be for enhancing or increasing cytotoxicity of the oncolytic virus in cancer or tumor cells.

[0187] In a further embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a degradation half-life greater than 20, 40, 60, 80, 100, 120, 240, 360 minutes or more in phosphate buffer at pH 7.4.

[0188] In still another embodiment, a compound as described herein may be a compound for which greater than about 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 90%, or greater, or any range bounded at a lower end by any one of these values, any range bounded at an upper end by any one of these values, or any range falling between any two of these values, of the compound remains after 3 hour incubation at 37° C. in aqueous, protein-rich Balb/c mouse plasma buffered 1:1 with pH 7.4 phosphate buffered saline (PBS). For example, a compound as described above may be a compound for which greater than or equal to ($\geq$) about 0.5% of the compound remains after 3 hour incubation at 37° C. in aqueous, protein-rich Balb/c mouse plasma buffered 1:1 with pH 7.4 phosphate buffered saline (PBS). For example, a compound as described herein may be a compound for which greater than or equal to ($\geq$) about 0.5% of the compound remains after 3 hour incubation at 37° C. in aqueous, protein-rich Balb/c mouse plasma buffered 1:1 with pH 7.4 phosphate buffered saline (PBS).

[0189] In another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a half-life greater than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400 minutes or more in a glutathione stability assay.

[0190] In still another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a lower LD₅₀ in the presence of an oncolytic virus than in its absence. In a further embodiment, the difference in LD₅₀ may be 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μ M or more in the presence of an oncolytic virus compared to its absence.

[0191] In still another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may be a compound having an LD₅₀ in the presence of virus which is greater than or equal to ($\geq$) about 1 μ m, 5 μ m, 10 μ m, 15 μ m, 20 μ m, 25 μ m, 30 μ m, 35 μ m, 40 μ m, 45 μ m, 50 μ m, 55 μ m, 60 μ m, 65 μ m, 70 μ m, 75 μ m, 80 μ m, 85 μ m, 90 μ m, 95 μ m, 100 μ m, or 105 μ m, or 110 μ m, or any range falling between any two of these values, less than (<) the LD₅₀ of the same compound in the absence of virus as determined in, for example, 786-0 cells where the virus is VSV.

[0192] In still another embodiment of any of the compounds, compositions, method or methods described above, the compound, or at least one of the compounds of the combination of compounds, may exhibit a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, or greater, or any range bounded at a lower end by any one of these values, any range bounded at an upper end by any one of these values, or any range falling between any two of these values, when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one. In one embodiment, for example, the compound may be a compound which exhibits a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is greater than or equal to ($\geq$) about 0.01 when reported as peak fold change in viral expression unit normalized to

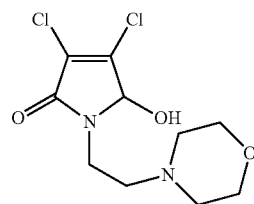
3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one. It will be understood by the person of skill in the art having regard to the teachings herein that in certain embodiments, PFC between two compounds need not always be calculated at the same compound concentration (although this may indeed be done). For example, PFC between two compounds could be determined at a dose of each compound which is experimentally determined to be particularly effective or near optimal, such as the most effective dose determined from a dose-response curve.

[0193] Thus, in a non-limiting embodiment, a compound as described above may be a compound which exhibits a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, or greater, or any range bounded at a lower end by any one of these values, any range bounded at an upper end by any one of these values, or any range falling between any two of these values, when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one (VSe1) taken with both compounds being used at the same concentration (for example, a particularly effective or near optimal VSe1 dose, such as the most effective dose of VSe1 determined from a dose-response curve), or taken with the compound and VSe1 being used at different concentrations (for example, a dose of each compound which is experimentally determined to be particularly effective or near optimal for each compound, such as the most effective dose for each compound determined from a dose-response curve).

[0194] In one embodiment, for example, the compound may be a compound which exhibits a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is greater than or equal to ($\geq$) about 0.01 when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one when both compounds are being used at the same concentration (for example, a particularly effective or near optimal VSe1 dose, such as the most effective dose of VSe1 determined from a dose-response curve), or when the compound and VSe1 are being used at different concentrations (for example, a dose of each compound which is experimentally determined to be particularly effective or near optimal for each compound, such as the most effective dose for each compound determined from a dose-response curve).

[0195] In still another embodiment of any of the method or methods described above, the method may further comprise administration of an anticancer agent or cancer therapeutic.

[0196] In another embodiment, there is provided herein a method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering VSe1 and MD03011,



(MD03011)

to said cells prior to, after, or concurrently with infection of the cells with the virus.

[0197] The present invention will be further illustrated in the following examples.

EXAMPLES

Example 1: Chemical and Biological
Characterization of Parent Compound VSe1
(Compound 1)

[0198] To gain a better understanding of the physiochemical nature of compound 1, we measured the compound's stability in phosphate buffer (pH=7.4) with an HPLC assay (FIG. 1B). Degradation occurred quickly ($t_{1/2}$ =28.8 min) leading to a complex mixture of products. Given the short half-life of compound 1 we considered that a degradation product in an aqueous media could be responsible for the impact on virus output from cancer cells, which can be observed up to at least 48h post-treatment with compound 1. To address this, we treated VSVΔ51-resistant 786-0 renal carcinoma cells with compound 1 and pre-incubated for up to 24 h in aqueous media. 40 h following infection with VSVΔ51 expressing luciferase (VSVΔ51-FLuc), virus output in viral expression units (VEUs) per ml was measured with a luciferase-reporter assay. Supporting compound 1 as an active agent as opposed to a degradation product, pre-incubation of compound 1 in media for as little as 90 minutes did not lead to robust enhancement of viral titers otherwise observed (FIG. 1C). However, removal of compound 1 following treatment at various times before infection with VSVΔ51-FLuc revealed that the induced effect of compound 1 on viral growth is rapid and sustained (FIG. 1D).

[0199] Analogue Synthesis and Evaluation

[0200] The above observations provided a rationale to derive novel compounds based on compound 1 with increased stability. Taking advantage of the versatile reactions mucohalic acids can undergo (FIG. 2), a diverse set of analogues was synthesized to reveal structure-activity relationships (SAR, see Table 1). Table 1 shows the structure activity and physico-chemical property relationship of VSe1 derivatives. Compounds were evaluated for viral sensitizer activity on VSV in 786-0 cells as described. Viral sensitizer activity is reported as Peak Fold Change (PFC) in Viral Expression Unit normalized to VSe1.^a Values in parenthesis (uM) indicates a particularly effective dose tested. LD50 indicates in vitro 50% lethal dose, which is provided with and without addition of virus.^b GSH half life was determined and is a measure of compound electrophilicity (low values indicate high electrophilicity) and stability. Stability in aqueous, protein-rich mouse plasma was also measured. A high % remaining after 3 hours indicates high stability. ^cNE indicates the compound did not detectably increase viral output. ^dND indicates not determined. ^e NR indicates not reactive.

[0201] Analogue compounds were screened for their ability to augment VSVΔ51-FLuc activity in 786-0 cells as above and their pro-viral activity was compared to compound 1. Cytotoxicity in presence and absence of virus was also assessed using an alamarBlue® metabolic dye. Analogue stability was measured with both a glutathione stability assay indicating physiochemical susceptibility to act as a Michael-acceptor, and a plasma assay indicating metabolic stability. Substitution of the β-chlorine with an alkyl amine resulted in compounds with dramatically increased stability but loss of viral enhancement activity (compounds 3, 4 (see Table 1)). Removal of the aryl group (compound 5) or replacement with a methoxide group (compound 6) resulted in active compounds with similar stability issues as com-

pound 1. Encouragingly, compounds with the 3,4-dichloro-5-hydroxy-1,5-dihydropyrrol-2-one scaffolds (compounds 9, 10) enhanced viral expression and showed remarkably improved stability with both the GSH and plasma assays. In addition, compound 10 was found to be stable over time in phosphate buffer (pH=7.4) (FIG. 3A). The LC/MS trace observed in the glutathione stability assay also showed that compound 10, contrary to compound 1, cleanly reacted with the nucleophile to form the glutathione adduct as the major detectable product (FIG. 3B). However, similarly to compound 1, the impact of compound 10 on viral growth was found to be rapid and sustained (FIG. 3C). In addition, both compounds 1 and 10 increased growth of oncolytic HSV-1 expressing GFP in 786-0 cancer cells as observed by fluorescence microscopy and standard plaque assay (FIG. 3D-E).

Given this information, we decided to further explore the 1,5-dihydropyrrol-2-one scaffold. A set of analogue compounds was synthesized to reveal further SAR (Table 1). Analogues with alterations to the hydroxyl group (compounds 11-15) reduced activity, as did alterations to the dichloro-moiety (compounds 16-21) when compared to compound 1. We then decided to investigate 3,4-dichloro-5-hydroxy-1,5-dihydropyrrol-2-one scaffold with various substitutions on the amine (Table 1). Analogues with saturated and unsaturated alkyl chains (compounds 22-27) were synthesized and evaluated which lead to the identification of cyclopropyl derivative compound 25, which displayed retained activity and remarkably improved toxicity and stability profiles. Morpholine containing analogue compound 28 also displayed promising activity with improved physiochemical properties. The phenylpropylamine derivative compound 30 showed improved activity over similar analogues with varying alkyl chain lengths (compounds 10, 29, 31). A set of aromatic and hetero-aromatic compounds were also evaluated (compounds 36-53), leading to the identification of compound 40, a 4-trifluoromethyl benzylamine derivative which displayed activity 1.6 fold greater than compound 1 in addition to improved stability.

[0202] Ex-Vivo Assessment of Analogue Activity

[0203] To facilitate evaluation of a larger number of compounds prior to in vivo testing, we chose to test a subset of analogues for their ability to enhance VSVΔ51 oncolysis in ex vivo tissue samples. Tissue samples from VSVΔ51 resistant CT26 murine colon cancer tumors as well as normal mouse brain, lung and spleens were core and put in tissue culture. Viable cores were selected for subsequent treatment with a compound and VSVΔ51 expressing green fluorescent protein (VSVΔ51-GFP). FIG. 4A shows representative images of infected cores that were pre-treated with a select dose of compound. Corresponding viral titers as determined by plaque assay are shown in FIGS. 4B-G. To varying extents, compound 1 and analogues enhanced VSVΔ51-GFP titers in CT26 colon cancer specimens. For compound 28 in particular, there was robust enhancement of VSVΔ51 output in the tumor specimens (nearly 100-fold) but little to no impact in normal tissue specimens, indicating that the specificity of VSVΔ51 towards tumor tissue is maintained following treatment with the compound.

[0204] In Vivo Evaluation

[0205] We next proceeded to evaluate the in vivo tolerability of a subset of analogues, selected based on desirable physiochemical characteristics, in vitro activity and ex vivo activity/selectivity profiles. Compounds were administered

intraperitoneally to Balb/c mice and body weight was monitored over several days. Mice were sacrificed when they reached the endpoint of 20% loss of body weight or showed significant outward signs of toxicity. FIG. 5 shows that compound 1 leads to toxicity starting at a dose of 10 mg/kg. In contrast analog compound 10 was well tolerated up to a dose of 50 mg/kg and analog compounds 24 and 28 up to 100 mg/kg.

[0206] Because it effectively and specifically enhanced OV output in tumors ex vivo and was very well tolerated in mice, we proceeded to evaluate analog compound 28 for its ability to increase the infection of tumors with VSVΔ51-FLuc in vivo. Balb/c mice were subcutaneously engrafted with CT26 cells and treated intra-tumorally with VSVΔ51-FLuc alone or in combination with analog compound 28. We used an in vivo imaging system (IVIS) to measure luciferase activity associated to virus replication 48 h post treatment. FIG. 6 shows that compared to VSVΔ51-FLuc alone, analog compound 28 (FIG. 6A-B) significantly enhanced tumor-specific viral replication specifically within the tumor. Similar results were obtained with analog compound 10 (FIGS. 6C-D).

[0207] In this study, we carried out SAR studies leading to the identification of new pyrrole derivatives of compound 1 with substantially improved stability, reduced electrophilicity, and retained or improved ability to enhance growth of OVs such as HSV-1 and VSVΔ51 in resistant cancer cell lines in vitro. In vivo evaluation further demonstrated that compounds such as analog 28 are exceptionally well tolerated (>10× the dose of parent compound 1) and effective at enhancing OV replication specifically within tumors. Ex vivo evaluation of compounds provided a useful pre-screening tool for selecting active and tumor-selective leads. In summary, we have derived a novel class of compounds that sensitize cells to virus infection and that could be useful for a broad range of applications utilizing viral vectors.

[0208] Experimental Methodology

[0209] Cell Lines

[0210] 786-0 (human renal carcinoma) and Vero (monkey kidney) cells were obtained from the American Type Culture Collection and maintained in Dulbecco's Modified Eagle's medium (Corning) supplemented with 10% fetal bovine serum and buffered with 30 mM Hepes. All cell lines were incubated at 37° C. with 5% CO₂.

[0211] Viruses

[0212] VSVΔ51 is a recombinant variant of the Indiana serotype of VSV harbouring a deletion of the 51st methionine in the M protein. VSVΔ51 expressing green fluorescent protein (GFP) or firefly luciferase (FLuc) are recombinant derivatives of VSVΔ51. All virus stocks were propagated in Vero cells, purified on Optiprep gradient and titered on Vero cells. HSV-1 N212 (an ICPO-deleted oncolytic strain) expressing GFP was obtained from Dr. Karen Mossman.

[0213] High-Throughput Screening of Analogs

[0214] High-throughput assessment of analog activity and cytotoxicity was performed as previously described (Garcia V, et al. J Vis Exp 2014). Briefly, stock preparations of compounds in dimethylsulfoxide (DMSO) were first diluted in 5% DMSO (in water) and then in cell culture media to obtain indicated concentration range. Diluted compounds were applied to confluent 786-0 cells in 96-well plates for indicated times prior to infection with VSVΔ51 (or mock) at a multiplicity of infection of 0.01. Vehicle alone (DMSO) was used as a negative control. 48 h later, infected and mock

infected cells were either incubated with Alamar Blue® according to manufacturer's instructions (Life Technologies) to assess viability or supernatants were collected for virus quantification by luciferase assay on confluent Vero cells in 96-well plates infected for 5-6 hours. To generate the standard curve, known amounts of virus (in plaque-forming units, or pfu) were added to the Vero cells at the same time as transfer of 786-0 supernatant. Upon measurement of bioluminescence, input pfu was plotted against mean relative light units for the standard curve, which was fit by four-parameter non-linear regression analysis. This standard curve was used to interpolate viral expression units (VEUs) which correlate with virus titer. For these experiments, compound 1 was used as a control and peak fold change (PFC) in VEU for each analog was normalized to peak PFC for VSe1 to generate the data in Table 1. Thus, all the data presented for analog compounds is in relation to results obtained for compound 1.

[0215] Glutathione Stability Experiment

[0216] 250 μL of a 40 mM DMSO stock solution of each compound was added to L-glutathione (15.4 mg, 5 mol equiv.) suspended in 250 μL of DMSO. The resulting mixture was placed in a 37° C. shaker. 10 μL aliquots were removed and quenched in 990 μL of water (containing 0.5% formic acid) at various time points, including at t=0 min, for analysis by ESI-LC-MS. All ESI-LC-MS analyses were collected on an API2000 LC/MS/MS System (Applied Biosystems) equipped with a turbo-ion spray ESI probe interfaced with a Prominence UFLC (Shimadzu) equipped with a reverse phase BDS Hypersil C18 50×2.1 mm column, particle size 3 μm (Thermo Scientific). HPLC/LCMS UV absorption was monitored at 254 nm and 210 nm. Both the compound and the glutathione adduct were identified by MS. Area of the UV peak was recorded for each time point.

[0217] Plasma Stability Assay

[0218] MRM Development. 10 mM stock solutions of each analogue were prepared in methanol and diluted with aqueous formic acid (0.1%) to a final concentration of 1 μM. 5 μL of the diluted solution was inserted into a Proxeon nanoelectrospray emitter (Thermo Scientific, Odense, Denmark) and analyzed in positive ion mode via nanoESI MS using a QStarXL hybrid quadrupole time-of-flight mass spectrometer (AB Sciex, Framingham, Mass., USA). Full mass and product ion spectra were collected for each compound using a nanoESI voltage of 1000 V, a declustering potential of 30 V and a focusing potential of 120 V. The product ion spectra were used to determine two multiple reaction monitoring (MRM) transitions for each compound with optimized collision energies: a "quantitative transition" to determine the relative quantities of each compound as well as a "confirmatory transition" to eliminate isobaric interference in the measurements.

[0219] Plasma Incubation. 1 mM stock solutions of each analogue were prepared in methanol and mixed with Balb/c mouse plasma (Innovative Research, Novi, Mich., USA) that was buffered 1:1 with PBS (pH=7.4). To increase the through-put of the assay, compounds were multiplexed in groups of three and analyzed in triplicate. The compounds were added to the plasma to a final concentration of 10 μM in a total volume of 400 μL. Immediately upon mixing, 200 μL of the sample mixture was quenched with 300 μL of aqueous formic acid (5%) to prevent degradation of the analogues. The remainder of the sample mixture was incubated at 37° C. for three hours and quenched in an identical

fashion. The quenched samples were passed through a 3 kDa Amicon molecular weight cut off filter (Millipore, Billerica, Mass., USA) by centrifugation at 14,000 rpm for 15 mins.

[0220] LC-MRM. 20 μ L of the filtrates was subjected to MRM analysis via a Qtrap 4000 (AB Sciex, Framingham, Mass., USA) hybrid triple quadrupole linear ion trap mass spectrometer with a Turbo V ion spray source coupled to a Dionex Ultimate3000 HPLC (Thermo Fisher Scientific, Waltham, Mass., USA) (see Supporting Information). Fritted fused silica columns (200 μ m ID) (Molex, Lisle, Ill., USA) were packed with 5 m Magic C18 (MICHROM Bioresources Inc., Auburn, Calif., USA) reversed phase beads to a length of 5 cm using an in house high-pressure vessel. Chromatographic separations were employed using reversed phase solvents (water and acetonitrile both containing 0.1% formic acid) over 10 minutes. Spectra were obtained using an ion spray voltage of 5000 V and a declustering potential of 25 V. Automatic quantitation was achieved using MultiQuant (AB Sciex, Framingham, Mass., USA) via integration of the peak areas for the extracted ion chromatogram of the quantitative MRM transition. The plasma stability of each compound was calculated as a percentage of the compound detected after three hours of incubation in plasma relative to the amount detected after immediate quenching.

[0221] Ex-Vivo Studies

[0222] Balb/c mice were implanted with CT26-WT (murine colon carcinoma) cells. Mice were sacrificed 24 days later, after tumors had reached at least 10 mm $\times$ 10 mm in size. Tumor, lung, spleen, brain, and abdominal muscle tissue were extracted from the mice, cut into 2 mm thick slices and cored into 2 mm $\times$ 2 mm pieces via punch biopsy. Each tissue core was incubated in 1 mL of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 30 mM HEPES and amphotericin B, in a 37 $^{\circ}$, 5% CO₂ humidified incubator. In order to assess the viability of each core, alamarBlue[®] was added to each well for a 4-hour incubation period. Viable cores were selected and treated with various concentrations of VSe1 and analogues. The cores were then infected with VSV Δ 51 expressing a GFP transgene (VSV Δ 51-GFP) four hours post treatment. GFP pictures were taken for each core 24 hours post infection. Cores and supernatants were collected 30 hours post infection and titered by plaque assay.

[0223] Further Analogue Evaluation

[0224] Synergistic Effect Studies

[0225] It was hypothesized that combinations of two or more compounds as described herein may also be used. An assay was conducted in which human renal carcinoma (786-0) cells were co-treated with VSe1 and the VSe1 analogue MD03011 (structure shown in Table 2). Assay results are provided in FIG. 7. As can be seen, a surprising synergist effect was observed when cells were treated with both VSe1 and MD03011, with fold change in VEU/mL climbing substantially. These results suggest that synergistic effects may be obtained using combinations of two or more compounds as described herein. Under the conditions tested, VSe1 combined with MD03011 provided an excellent synergistic effect.

[0226] Infection of Cells with Oncolytic Maraba Virus

[0227] FIG. 8 shows that VSe1 and its analogues may enhance infection of cells with oncolytic Maraba virus. Human renal carcinoma (786-0) cells were left untreated or, treated with compound at various concentrations. After 4

hours, cells were infected with MG1-eGFP at MOI 0.005. eGFP fluorescence was detected 24 h after MG1 infection and quantified using a Cellomics ArrayScan high content screening microscope. Compounds tested include compounds 1, 10, 27, 28, and 29 as shown in Table 1, each of which showed increased GFP counts in this assay.

[0228] Infection of Cells with Herpes Simplex Virus-1

[0229] FIG. 9 shows that VSe1 and its analogs enhance infection of cells with Herpes Simplex Virus-1. Human renal carcinoma (786-0) cells were left untreated or, treated with compound at various concentrations. After 4 hours, cells were infected with ICPO-null HSV-N212eGFP at MOI 0.01. eGFP fluorescence was detected 48 h after HSV infection and quantified using a Cellomics ArrayScan high content screening microscope. Compounds tested include compounds 1, 10, 27, 28, and 29 as shown in Table 1, each of which showed increased GFP counts in this assay.

[0230] VSe1 Analogue Effect on Therapeutic Activity of Oncolytic VSV in a Cancer Xenograft Model

[0231] FIG. 10 shows that VSe1 analogue 28 (see structure shown in Table 1) enhances therapeutic activity of oncolytic VSV in a human colon cancer xenograft model. FIG. 10(A) shows results obtained when VSV Δ 51-resistant HT29 cells (human colon carcinoma) were subcutaneously engrafted into female Balb/c mice, and the xenograft mice were then treated with compound 28 by intratumoral injection followed by VSV Δ 51-FLuc. Mouse survival was monitored over time, as shown in FIG. 10(B). These results demonstrate that compound 28 reduced tumor growth, and increased mouse survival rates, in these experimental conditions.

[0232] All citations are hereby incorporated by reference.

[0233] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

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TABLE 1

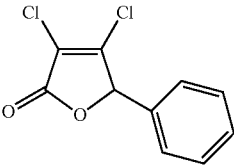
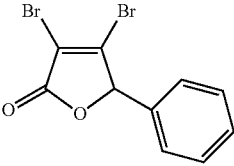
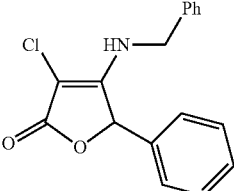
Compound	Structure	Normalized PFC			GSH	Plasma stab.
		(PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	half-life (min) ^b	% remanent at 3 hrs
1		1.00 (60 μM)	79	16	<5	0
2		0.37 (72 μM)	87	50	<5	0
3		0.19 (96 μM)	140	140	NR ^c	65.6 ± 6.5

TABLE 1-continued

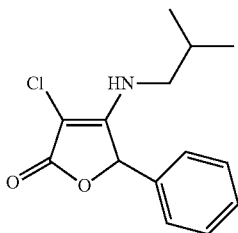
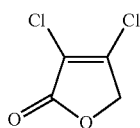
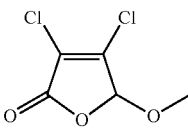
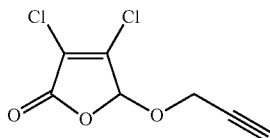
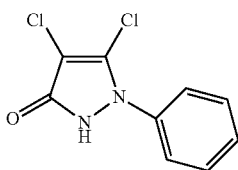
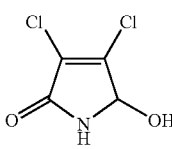
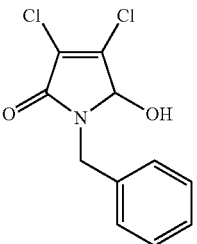
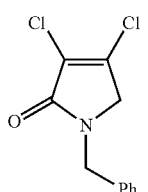
Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
4		0.18 (80 μM)	90	90	NR	0
5		0.27 (36 μM)	41	27	<5	0
6		0.21 (60 μM)	73	51	<5	0
7		0.17 (60 μM)	52	17	<5	0
8		NE ^c	>180	>180	NR	88.3 $\pm$ 9.3
9		0.67 (120 μM)	148	87	117	0
10		0.37 (48 μM)	67	51	32	19.8 $\pm$ 0.4
11		0.03 (240 μM)	332	332	64	42.5 $\pm$ 9.6

TABLE 1-continued

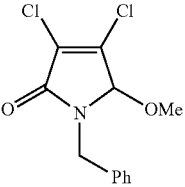
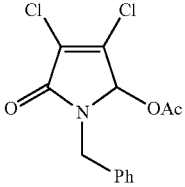
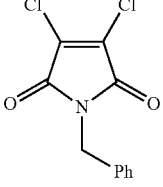
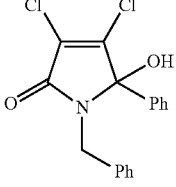
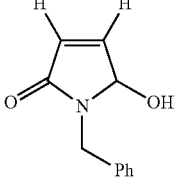
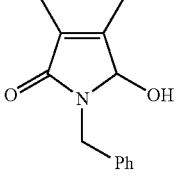
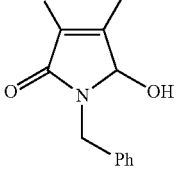
Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
12		0.28 (180 μM)	206	203	118	47.6 $\pm$ 1.4
13		0.16 (60 μM)	61	45	21	0
14		0.03 (17.8 μM)	104	98	<5	70.2 $\pm$ 8.4
15		NE	66	66	340	14.9 $\pm$ 7.1
16		NE	>360	>360	NR ^e	98.2 $\pm$ 3.7
17		NE	>360	>360	NR	82.0 $\pm$ 10.2
18		NE	>360	>360	—	ND ^d

TABLE 1-continued

Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
19		NE	250	245	—	ND
20		NE	>360	>360	NR	102.9 $\pm$ 1.6
21		NE	>90	>90	NR	102.7 $\pm$ 10.8
22		0.47 (96 μM)	119	76	68	72.0 $\pm$ 3.0
23		0.06 (72 μM)	>90	55	—	ND
24		0.48 (120 μM)	174	96	61	91.6 $\pm$ 5.2
25		0.74 (80 μM)	127	51	53	54.8 $\pm$ 3.6

TABLE 1-continued

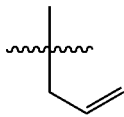
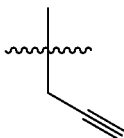
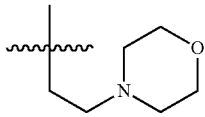
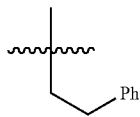
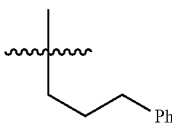
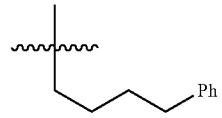
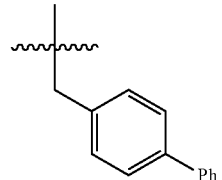
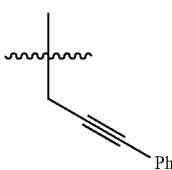
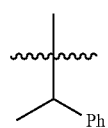
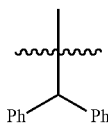
Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
26		0.52 (96 μM)	110	66	46	64.8 $\pm$ 7.7
27		0.04 (80 μM)	100	60	21	9.0 $\pm$ 1.4
28		1.00 (80 μM)	153	55	96	38.9 $\pm$ 5.2
29		0.51 (72 μM)	74	27	74	57.6 $\pm$ 6.6
30		0.57 (32 μM)	36	20	50	42.9 $\pm$ 7.2
31		0.26 (40 μM)	40	34	72	40.1 $\pm$ 9.8
32		0.11 (27 μM)	28	5	24	ND
33		0.30 (18 μM)	18	12	24	0
34		0.33 (72 μM)	74	6	31	48.0 $\pm$ 16.5
35		0.14 (27 μM)	36	23	43	63.8 $\pm$ 3.2

TABLE 1-continued

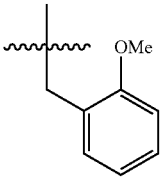
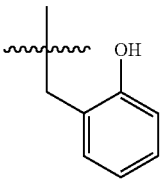
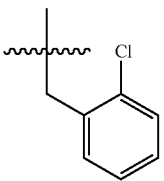
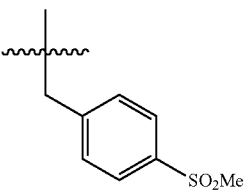
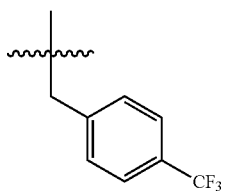
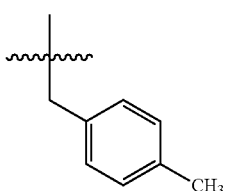
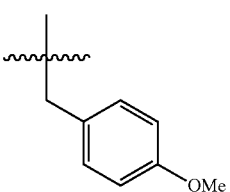
Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
36		0.07 (180 μM)	>180	>180	34	28.2 $\pm$ 2.6
37		0.56 (48 μM)	58	38	41	0.7 $\pm$ 0.1
38		0.35 (216 μM)	215	107	32	25.7 $\pm$ 2.9
39		0.51 (60 μM)	>90	25	34	41.4 $\pm$ 5.4
40		1.6 (27 μM)	36	13	32	15.3 $\pm$ 2.5
41		0.19 (40 μM)	39	30	35	51.4 $\pm$ 8.2
42		0.03 (40 μM)	55	17	40	49.1 $\pm$ 12.4

TABLE 1-continued

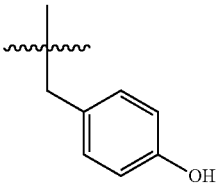
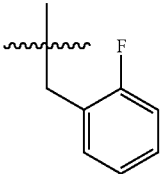
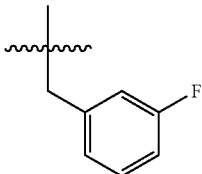
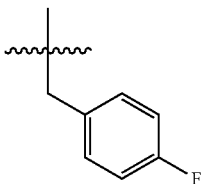
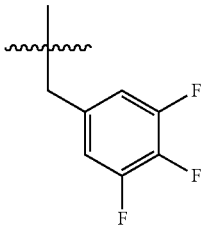
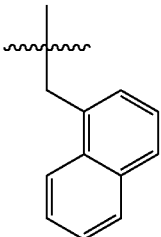
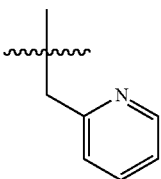
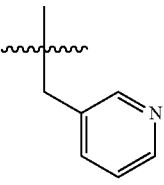
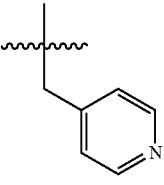
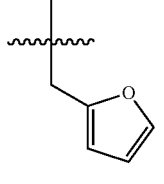
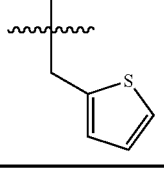
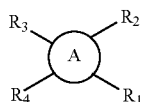
Compound	Structure	Normalized PFC (PFC dose (μM)) ^a	LD50 (μM)	LD50 with virus (μM)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
43		0.15 (60 μM)	>90	45	69	58.3 $\pm$ 0.6
44		0.08 (60 μM)	63	39	31	45.9 $\pm$ 8.1
45		0.06 (48 μM)	43	37	31	54.2 $\pm$ 4.2
46		0.09 (40 μM)	42	36	32	23.1 $\pm$ 0.8
47		0.10 (40 μM)	36	35	35	22.7 $\pm$ 8.4
48		0.65 (32 μM)	38	24	14	36.5 $\pm$ 7.6
49		0.31 (96 μM)	131	67	64	44.6 $\pm$ 1.2

TABLE 1-continued

Compound	Structure	Normalized PFC (PFC dose (μ M)) ^a	LD50 (μ M)	LD50 with virus (μ M)	GSH half-life (min) ^b	Plasma stab. % remaining at 3 hrs
50		0.42 (60 μ M)	85	29	54	39.6 $\pm$ 2.6
51		0.31 (72 μ M)	89	28	53	44.0 $\pm$ 1.0
52		0.25 (60 μ M)	67	28	45	54.1 $\pm$ 5.1
53		0.51 (60 μ M)	64	27	40	50.1 $\pm$ 10.5

1. A method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (I):



(I)

an N-oxide, pharmaceutically acceptable addition salt, quaternary amine or stereochemically isomeric form thereof, wherein:

A is a 5-membered heterocyclic ring comprising 0 or 1 double bond and 1 heteroatom selected from O, substituted or unsubstituted N;

R¹ and R⁴ are each independently H, oxo, hydroxyl, alkynyloxy, phenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, or indolyl;

R² and R³ are each independently hydrogen, halogen, alkynylamino, isobutylamino, or benzylamino;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

2. A method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer

or tumor cells, comprising administering a compound, or a combination of compounds, selected from the group consisting of:

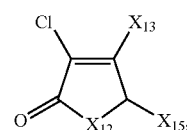
α,β -dichloro- γ -hydroxy-N-benzyl-crotonic lactam; 3,4-dichloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dibromo-5-prop-2-ynyloxy-5H-furan-2-one; 3-chloro-5-phenyl-4-prop-2-ynylamino-5H-furan-2-one; 3-chloro-4-isobutylamino-5-prop-2-ynyloxy-5H-furan-2-one; 1-benzyl-3,4-dichloro-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-(2-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one; 4-benzylamino-3-chloro-5-prop-2-ynyloxy-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-prop-2-ynyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5H-furan-2-one; benzo[1,3]dioxole-5,6-dione; 4,5-dichloro-2H-pyridazin-3-one; 4,5-dichloro-2-phenyl-2H-pyridazin-3-one; 3,4-dichloro-1-phenyl-pyrrole-2,5-dione; 3,4-dichloro-5-(1H-indol-3-yl)-5H-furan-2-one, indole-3-crotonic acid; 3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 5-phenyl-4,5-dihydro-3aH-pyrrolo[1,2-a]quinolin-1-one; 3,4-dichloro-5-(3-nitro-phenyl)-5H-furan-2-one; 3,4-dichloro-5-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-prop-2-ynyl-5-prop-2-ynyloxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-1-(2-chloro-benzyl)-5-hydroxy-1,5-dihydro-pyrrol-2-one; 3,4-dichloro-5-hydroxy-1-propyl-1,5-dihydro-pyrrol-2-one; 1-phenyl-pyrrole-2,5-dione; 3,4-

dichloro-1-propyl-pyrrole-2,5-dione; 1-benzyl-3,4-dichloro-pyrrole-2,5-dione; 3,4-dichloro-5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one; 1-(1-benzyl-1H-[1,2,3]triazol-4-ylmethyl)-3,4-dichloro-5-hydroxy-1,5-dihydro-pyrrol-2-one; [4-(4-chloro-3-isobutylamino-5-oxo-2,5-dihydro-furan-2-yloxymethyl)-[1,2,3]triazol-1-yl]-acetic acid; [4-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-pyrrol-1-ylmethyl)-[1,2,3]triazol-1-yl]-acetic acid; 3,4-dichloro-5-hydroxy-1-phenethyl-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-morpholinoethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-cyclopropyl-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-mercaptoethyl)-1H-pyrrol-2(5H)-one; 2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethanaminium 2,2,2-trifluoroacetate; 3,4-dichloro-5-hydroxy-1-(3-phenylprop-2-ynyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(trifluoromethyl)benzyl)-1H-pyrrol-2(5H)-one; 1-(biphenyl-4-ylmethyl)-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-nitrobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-methoxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(2-chlorobenzyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 1-benzhydryl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(naphthalen-1-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(1-phenylethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-3-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-4-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(pyridin-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-(furan-2-ylmethyl)-5-hydroxy-1H-pyrrol-2(5H)-one; N-(2-(3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide; (3aS)-2,3-dichloro-5-phenyl-4,5-dihydropyrrolo[1,2-a]quinolin-1(3aH)-one; 3,4-diiodo-2-phenyl-2,5-dihydrofuran; D-Gluconamide, N-octyl; (S)-11-amino-4,7,10,14-tetraoxo-15-((2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-3,6,9,13-tetraazapentadecan-1-oic acid; 1-allyl-3,4-dichloro-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(2-hydroxybenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(thiophen-2-ylmethyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-(methylsulfonyl)benzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-1-((4,5-dimethyloxazol-2-yl)methyl)-5-hydroxy-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(3,4,5-trifluorobenzyl)-1H-pyrrol-2(5H)-one; 3,4-dichloro-5-hydroxy-1-(4-methoxybenzyl)-1H-pyrrol-2(5H)-one; 4,5-dichloro-2-(2,2,2-trifluoroethyl)pyridazin-3(2H)-one; 4,5-dichloro-2-cyclohexylpyridazin-3(2H)-one; methyl 2-((3,4-dichloro-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate; 4,5-dichloro-2-o-tolylpyridazin-3(2H)-one; 4,5-dichloro-2-(2-(dimethylamino)ethyl)pyridazin-3(2H)-one hydrochloride; and 4,5-dichloro-2-(4-fluorophenyl)pyridazin-3(2H)-one or any compound or group of compounds defined in Table 1;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

3. A method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer

or tumor cells, comprising administering a compound, or a combination of compounds, defined by formula (VIII):



(VIII)

or

a pharmaceutically acceptable salt, or stereochemically isomeric form thereof, wherein:

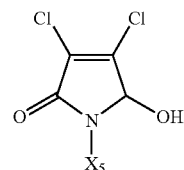
X₁₂ is O, NH, or substituted N;

X₁₃ is halogen such as Cl, or NHX₁₄, wherein X₁₄ is a substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl; and

X₁₅ is H, OH, =O, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl such as substituted or unsubstituted phenyl, or OX₁₆, wherein X₁₆ is H, linear or branched substituted or unsubstituted alkyl, alkenyl, alkynyl, or acyl, or X₁₆ is acetyl, methyl, or —CH₂—C≡CH;

to said cells prior to, after, or concurrently with infection of the cells with the virus.

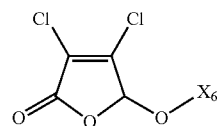
4. The method according to claim 3, wherein the compound, or at least one of the compounds of the combination of compounds, is defined by formula (III):



(III)

wherein X₅ is H, substituted or unsubstituted linear or branched C₁-C₁₂ alkyl, alkenyl, or alkynyl, substituted or unsubstituted mono- or bi-cycloaryl or -heteroaryl, substituted or unsubstituted cycloalkyl or heterocycloalkyl, or wherein X₅ is substituted or unsubstituted alkyloxy, phenyl, alkylphenyl, substituted phenyl, benzyl, substituted benzyl, triazolyl, substituted triazolyl, naphthalenyl, substituted naphthalenyl, substituted or unsubstituted pyridinyl, substituted or unsubstituted furanyl or thiofuranyl, thiophenyl, sulfonobenzyl, methylsulfonobenzyl, pyrrolyl, substituted or unsubstituted morpholine, cycloalkyl, alkylthiol, substituted or unsubstituted alkyamine, or substituted or unsubstituted oxazoline.

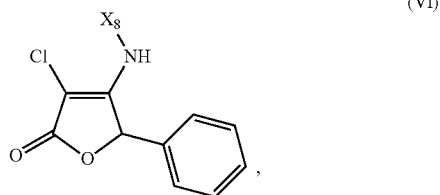
5. The method according to claim 3, wherein the compound, or at least one of the compounds of the combination of compounds, is defined by formula (IV):



(IV)

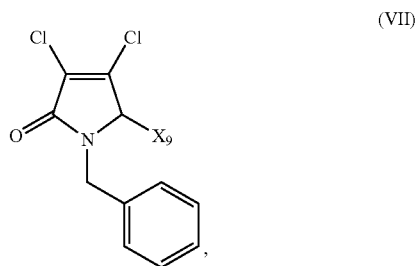
wherein X_6 is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or wherein X_6 is substituted or unsubstituted methyl, alkyl triazolyl, acetyl, or $-\text{CH}_2-\text{C}\equiv\text{CH}$.

6. The method according to claim 3, wherein the compound, or at least one of the compounds of the combination of compounds, is defined by formula (VI):



wherein X_8 is substituted or unsubstituted linear or branched alkyl, alkenyl, or alkynyl, or substituted or unsubstituted aryl or heteroaryl, or X_8 is substituted or unsubstituted benzyl.

7. The method according to claim 3, wherein the compound, or at least one of the compounds of the combination of compounds, is defined by formula (VII):



wherein X_9 is H, OH, OX_{11} , or $=\text{O}$, wherein X_{11} is H, substituted or unsubstituted linear or branched alkyl, alkenyl, alkynyl, or acyl, or X_{11} is acetyl, methyl, or $-\text{CH}_2-\text{C}\equiv\text{CH}$.

8-10. (canceled)

11. The method of claim 1, wherein the cells are cancer cells in vivo, or in vitro.

12. The method of claim 11 wherein the in vivo cancer cells are from a mammalian subject.

13. The method of claim 8, wherein the mammalian subject is a human subject.

14. A method of increasing the oncolytic activity of an oncolytic virus in cancer or tumor cells, comprising administering a compound, or a combination of compounds, as defined in claim 1 to said cancer or tumor cells prior to, concurrently with or after the oncolytic virus.

15. The method of claim 14, wherein the cancer cells are in vivo, or in vitro.

16. The method of claim 14 wherein the in vivo cancer cells are from a mammalian subject.

17. The method of claim 16, wherein the mammalian subject is a human subject.

18-21. (canceled)

22. The method of claim 1, wherein the compound, or at least one of the compounds of the combination of compounds, exhibits a degradation half-life greater than 20, 40, 60, 80, 100, 120, 240, 360 minutes or more in phosphate buffer at pH 7.4.

23. The method of claim 1, wherein the compound, or at least one of the compounds of the combination of compounds, exhibits a half-life greater than 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400 minutes or more in a glutathione stability assay.

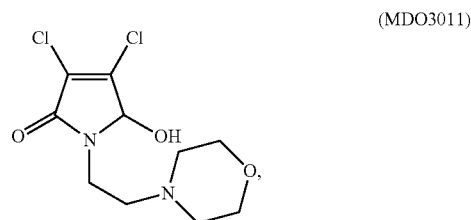
24. The method of claim 1, wherein the compound, or at least one of the compounds of the combination of compounds, exhibits a lower LD50 in the presence of an oncolytic virus than its absence.

25. The method of claim 24, wherein the difference in LD50 is 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μM or more in the presence of an oncolytic virus compared to its absence.

26. The method of claim 1, wherein the compound, or at least one of the compounds of the combination of compounds, has a viral sensitizer activity on VSV Δ 51 in 786-0 cells which is about 0.01 or greater when reported as peak fold change in viral expression unit normalized to 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-one.

27. The method of claim 1, wherein the method further comprises administration of an anticancer agent or cancer therapeutic.

28. A method of enhancing or increasing the infection, spread, titer, or cytotoxicity of an oncolytic virus in cancer or tumor cells, comprising administering VSe1 and MD03011,



to said cells prior to, after, or concurrently with infection of the cells with the virus.

* * * * *