INHIBITORS OF INFLUENZA VIRUSES REPLICATION

CROSS REFERENCE TO RELATED APPLICATION

[0001] This PCT application claims the benefit of U.S. provisional application no. 61/903,572, filed on November 13, 2013. This document is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to compounds and solid forms of compounds that are useful for inhibiting influenza virus replication, treating or reducing the severity of influenza infections in patients, and prophylactically preventing or reducing the incidence of influenza infections in patients.

BACKGROUND

[0003] Influenza spreads around the world in seasonal epidemics, resulting in the deaths of hundreds of thousands annually - millions in pandemic years. For example, three influenza pandemics occurred in the 20th century and killed tens of millions of people, with each of these pandemics being caused by the appearance of a new strain of the virus in humans. Often, these new strains result from the spread of an existing influenza virus to humans from other animal species.

[0004] Influenza is primarily transmitted from person to person via large virus-laden droplets that are generated when infected persons cough or sneeze; these large droplets can then settle on the mucosal surfaces of the upper respiratory tracts of susceptible individuals who are near (e.g. within about 6 feet) infected persons. Transmission might also occur through direct contact or indirect contact with respiratory secretions, such as touching surfaces contaminated with influenza virus and then touching the eyes, nose or mouth. Adults might be able to spread influenza to others from 1 day before getting symptoms to approximately 5 days after symptoms start. Young children and persons with weakened immune systems might be infectious for 10 or more days after onset of symptoms.

[0005] Influenza viruses are RNA viruses of the family Orthomyxoviridae, which comprises five genera: Influenza virus A, Influenza virus B, Influenza virus C, ISA virus and Thogoto virus.

[0006] The Influenza virus A genus has one species, influenza A virus. Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics. The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease. The influenza A virus

can be subdivided into different serotypes based on the antibody response to these viruses. The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are: H1N1 (which caused Spanish influenza in 1918), H2N2 (which caused Asian Influenza in 1957), H3N2 (which caused Hong Kong Flu in 1968), H5N1 (a pandemic threat in the 2007–08 influenza season), H7N7 (which has unusual zoonotic potential), H1N2 (endemic in humans and pigs), H9N2, H7N2 , H7N3 and H10N7.

[0007] The Influenza virus B genus has one species, influenza B virus. Influenza B almost exclusively infects humans and is less common than influenza A. The only other animal known to be susceptible to influenza B infection is the seal. This type of influenza mutates at a rate 2–3 times slower than type A and consequently is less genetically diverse, with only one influenza B serotype. As a result of this lack of antigenic diversity, a degree of immunity to influenza B is usually acquired at an early age. However, influenza B mutates enough that lasting immunity is not possible. This reduced rate of antigenic change, combined with its limited host range (inhibiting cross species antigenic shift), ensures that pandemics of influenza B do not occur.

[0008] The Influenza virus C genus has one species, influenza C virus, which infects humans and pigs and can cause severe illness and local epidemics. However, influenza C is less common than the other types and usually seems to cause mild disease in children.

[0009] Influenza A, B and C viruses are very similar in structure. The virus particle is 80-120 nanometers in diameter and usually roughly spherical, although filamentous forms can occur. Unusual for a virus, its genome is not a single piece of nucleic acid; instead, it contains seven or eight pieces of segmented negative-sense RNA. The Influenza A genome encodes 11 proteins: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1, NS2(NEP), PA, PB1, PB1-F2 and PB2.

[0010] HA and NA are large glycoproteins on the outside of the viral particles. HA is a lectin that mediates binding of the virus to target cells and entry of the viral genome into the target cell, while NA is involved in the release of progeny virus from infected cells, by cleaving sugars that bind the mature viral particles. Thus, these proteins have been targets for antiviral drugs. Furthermore, they are antigens to which antibodies can be raised. Influenza A viruses are classified into subtypes based on antibody responses to HA and NA, forming the basis of the H and N distinctions (*vide supra*) in, for example, H5N1.

[0011] Influenza produces direct costs due to lost productivity and associated medical treatment, as well as indirect costs of preventative measures. In the United States, influenza is responsible for a total cost of over $10 billion per year, while it has been estimated that a

future pandemic could cause hundreds of billions of dollars in direct and indirect costs. Preventative costs are also high. Governments worldwide have spent billions of U.S. dollars preparing and planning for a potential H5N1 avian influenza pandemic, with costs associated with purchasing drugs and vaccines as well as developing disaster drills and strategies for improved border controls.

[0012] Current treatment options for influenza include vaccination, and chemotherapy or chemoprophylaxis with anti-viral medications. Vaccination against influenza with an influenza vaccine is often recommended for high-risk groups, such as children and the elderly, or in people that have asthma, diabetes, or heart disease. However, it is possible to get vaccinated and still get influenza. The vaccine is reformulated each season for a few specific influenza strains but cannot possibly include all the strains actively infecting people in the world for that season. It may take six months for the manufacturers to formulate and produce the millions of doses required to deal with the seasonal epidemics; occasionally, a new or overlooked strain becomes prominent during that time and infects people although they have been vaccinated (as by the H3N2 Fujian flu in the 2003-2004 influenza season). It is also possible to get infected just before vaccination and get sick with the very strain that the vaccine is supposed to prevent, as the vaccine may require several weeks to become effective.

[0013] Further, the effectiveness of these influenza vaccines is variable. Due to the high mutation rate of the virus, a particular influenza vaccine usually confers protection for no more than a few years. A vaccine formulated for one year may be ineffective in the following year, since the influenza virus changes rapidly over time, and different strains become dominant.

[0014] Also, because of the absence of RNA proofreading enzymes, the RNA-dependent RNA polymerase of influenza vRNA makes a single nucleotide insertion error roughly every 10 thousand nucleotides, which is the approximate length of the influenza vRNA. Hence, nearly every newly-manufactured influenza virus is a mutant-antigenic drift. The separation of the genome into eight separate segments of vRNA allows mixing or reassortment of vRNAs if more than one viral line has infected a single cell. The resulting rapid change in viral genetics produces antigenic shifts and allows the virus to infect new host species and quickly overcome protective immunity.

[0015] Antiviral drugs can also be used to treat influenza, with neuraminidase inhibitors being particularly effective, but viruses can develop resistance to the standard antiviral drugs. Such agents can be prepared so as to have a variety of different chemical forms including

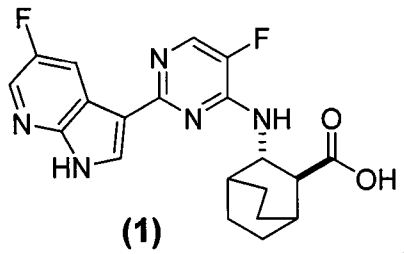
chemical derivatives or salts, or to have different physical forms. For example, they may be amorphous, may have different crystalline polymorphs, or may exist in different solvation or hydration states. By varying the forms, it may be possible to vary the physical properties thereof. Such different forms may have different properties, in particular, as oral formulations. Specifically, it may be desirable to identify improved forms that exhibit improved properties, such as increased aqueous solubility and stability, better processability or preparation of pharmaceutical formulations, and increase of the bioavailability of orally-administered compositions. Such improved properties discussed above may be altered in a way that is beneficial for a specific therapeutic effect.

[0016] Variation of the forms of an antiviral agent can be one of many ways in which to modulate the physical properties of such antiviral agent to be more useful in treating influenza.

SUMMARY OF THE INVENTION

[0017] The present invention generally relates to polymorphic forms of Compound (**1**) or a pharmaceutically acceptable salt thereof, to pharmaceutically acceptable formulations thereof, methods of preparing such polymorphic forms of Compound (**1**), and to uses of such polymorphic forms for inhibiting the replication of influenza viruses, for reducing the amount of influenza viruses, and for treating influenza.

[0018] In one embodiment, the present invention is directed to a polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof, wherein Compound (**1**) is represented by the following structural formula:



(1)

and wherein the polymorphic form is selected from the group consisting of: Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), and Form A of tosylate salt of Compound (**1**).

[0019] In another embodiment, the present invention is directed to a pharmaceutically acceptable formulation comprising a polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof disclosed herein and at least one pharmaceutically acceptable carrier or excipient.

[0020] In yet another embodiment, the present invention is directed to a method of inhibiting the replication of influenza viruses in a biological *in vitro* sample or in a patient. The method comprises administering to the sample an effective amount of a polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof disclosed herein.

[0021] In yet another embodiment, the present invention is directed to a method of reducing the amount of influenza viruses in a biological *in vitro* sample or in a patient. The method comprises administering to the sample an effective amount of a polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof disclosed herein.

[0022] In yet another embodiment, the present invention is directed to a method of treating influenza in a patient. The method comprises administering to the sample an effective amount of a polymorph form of Compound (**1**) or a pharmaceutically acceptable salt thereof disclosed herein.

[0023] In yet another embodiment, the present invention is directed to a method of preparing Form A of HCl salt of Compound (**1**)•1/2H_2O. The method comprises mixing HCl with Compound (**1**) in a solvent system that includes water and one or more organic solvents, wherein the solvent system has a water activity of 0.05-0.85. Compound (**1**) may be solvated or non-solvated, and/or amorphous or crystalline.

[0024] In yet another embodiment, the present invention is directed to a method of preparing Form F of HCl salt of Compound (**1**)•3H_2O. The method comprises: mixing HCl and Compound (**1**) in a solvent system that includes water or that includes water and one or more organic solvents, wherein the solvent system has a water activity of equal to, or greater than, 0.9, such as 0.9-1.0; or stirring Form A of HCl salt of Compound (**1**)•1/2H_2O in a solvent system that includes water or that includes water and one or more organic solvents, wherein the solvent system has a water activity of equal to, or greater than, 0.9, such as 0.9-1.0. Compound (**1**) may be solvated or non-solvated, and/or amorphous or crystalline.

[0025] In yet another embodiment, the present invention is directed to a method of preparing Form D of HCl salt of Compound (**1**). The method comprises dehydrating Form A of HCl salt of Compound (**1**)•1/2H_2O.

[0026] In yet another embodiment, the present invention is directed to a method of preparing Form A of Compound (**1**). The method comprises stirring an amorphous Compound (**1**) or a solvate of Compound (**1**) in a solvent system that includes water and ethanol.

[0027] In yet another embodiment, the present invention is directed to a method of preparing Form A of tosylate salt of Compound (**1**). The method comprises stirring a mixture of an

amorphous Compound (**1**) or a solvate of Compound (**1**), *p*-toluenesulfonic acid, and a solvent system that includes acetonitrile.

[0028] A 2-Methyl THF solvate of Compound (**1**) is also encompassed in the invention.

[0029] In yet another embodiment, the invention is directed to a method of reducing the amount of influenza viruses in a biological *in vitro* sample or in a subject, comprising administering to the sample an effective amount of a polymorphic form of Compound (**1**) disclosed herein.

[0030] In yet another embodiment, the invention is directed to a method of inhibiting the replication of influenza viruses in a biological *in vitro* sample or in a subject, comprising administering to the sample an effective amount of a polymorphic form of Compound (**1**) disclosed herein.

[0031] In yet another embodiment, the invention is directed to a method of treating influenza in a subject, comprising administering to the subject a therapeutically effective amount of a polymorphic form of Compound (**1**) disclosed herein.

[0032] The invention also includes uses of polymorphic forms of Compound (**1**) disclosed herein for inhibiting the replication of influenza viruses, for reducing the amount of influenza viruses, or treating influenza, in a subject. The invention also includes uses of a polymorphic form of Compound (**1**) disclosed herein for the manufacture of a medicament for inhibiting the replication of influenza viruses, for reducing the amount of influenza viruses, or treating influenza, in a subject.

[0033] In yet another aspect, the present invention is directed to a dosage regimen of Compound (**1**) or a pharmaceutically acceptable salt thereof (e.g., Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), and Form A of tosylate salt of Compound (**1**)) in a range of 100 mg to 1,600 mg.

BRIEF DESCRIPTION OF DRAWINGS

[0034] FIGs 1 and 2 are a X-ray powder diffraction (XRPD) pattern and C^{13} solid state nuclear magnetic spectroscopy (C^{13} SSNMR) spectrum of Form A of HCl salt of Compound (**1**)•1/2H_2O, respectively.

[0035] FIGs 3 and 4 are a XRPD pattern and C^{13} SSNMR spectrum of Form F of HCl salt of Compound (**1**)•3H_2O, respectively.

[0036] FIGs 5 and 6 are a XRPD pattern and C^{13} SSNMR spectrum of Form D of HCl salt of Compound (**1**), respectively.

[0037] FIGs 7 and 8 are XRPD pattern and C^{13} SSNMR spectrum of Form A of Compound

(1), respectively.

[0038] FIG. 9 is a XRPD pattern of Form A of tosylate salt of Compound (1).

[0039] FIG. 10 is a XRPD pattern of a 2-methyltetrahydrofuran (2-MeTHF) solvate of Compound (1).

[0040] FIG. 11 is a XRPD pattern of an amorphous form of Compound (1).

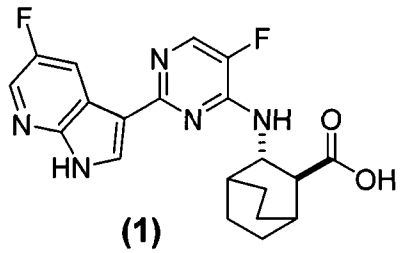
[0041] FIG. 12 is phase diagram of temperature against water activity for the transition among different polymorphs of an HCl salt of Compound (1).

[0042] FIG. 13 is a graph showing AUC viral shedding for 1200 mg/600 mg of Form A of HCl salt of Compound (1)•1/2H_2O dose group in a live, attenuated influenza challenge model in humans.

DETAILED DESCRIPTION OF THE INVENTION

[0043] I. SOLID FORMS

[0044] Compound (1) represented by the following structural formula:



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and pharmaceutically acceptable salts thereof can inhibit the replication of influenza viruses and also described in WO 2010/148197.

[0045] Compound (1) can exist in or form different polymorphic forms. As known in the art, polymorphism is an ability of a compound to crystallize as more than one distinct crystalline or "polymorphic" species. A polymorph is a solid crystalline phase of a compound with at least two different arrangements or polymorphic forms of that compound molecule in the solid state. Polymorphic forms of any given compound are defined by the same chemical formula or composition and are as distinct in chemical structure as crystalline structures of two different chemical compounds. Generally, different polymorphs can be characterized by analytical methods such as X-ray powder diffraction (XRPD) pattern, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC), or by its melting point, or other techniques known in the art. As used herein, the term "polymorphic form" includes solvates and neat polymorphic form that does not have any solvates.

[0046] As used herein, "Compound (1)" means the free base form of Compound (1). Accordingly, "HCl salt of Compound (1)" means a HCl salt of the free base compound, and "tosylate salt of Compound (1)" means a tosylate salt of the free base compound. It is noted

that Compound (**1**) and salts of Compound (**1**) can be solvated or non-solvated unless specified otherwise. Also, it is noted Compound (**1**) and salts of Compound (**1**) can be crystalline or amorphous unless specified otherwise.

[0047] In one embodiment, the present invention is directed to polymorphic Form A of HCl salt of Compound (**1**)•1/2H_2O. This form is a polymorphic form of HCl salt of Compound (**1**) that includes water as a solvate in a half equivalent per Compound (**1**). In one specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 10.5, 5.2, 7.4, and 18.9 (± 0.2 degrees) in an X-ray powder diffraction pattern. In another specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 25.2 ± 0.2, 16.5 ± 0.2, 18.1 ± 0.2, and 23.0 ± 0.2 in an X-ray powder diffraction pattern. In another specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the following positions listed in Table 2. In yet another specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized as having an XRPD pattern substantially the same as that shown in FIG. 1. The XRPD patterns are obtained at room temperature using Cu K alpha radiation. In yet another specific embodiment, the polymorphic Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized as having one or more characteristic peaks at 29.2, 107.0, 114.0, and 150.7 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, the polymorphic Form A of HCl salt of Compound (**1**)•1/2H_2O is further characterized as having one or more characteristic peaks at 22.1, 24.6, 47.7, and 54.8 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized as having C^{13} SSNMR peaks listed in Table 3. In yet another specific embodiment, Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized as having a C^{13} SSNMR spectrum substantially the same as that shown in FIG. 2.

[0048] In one embodiment, the present invention is directed to polymorphic Form F of HCl salt of Compound (**1**)•3H_2O. This form is a polymorphic form of HCl salt of Compound (**1**) that includes water as a solvate in three equivalents per Compound (**1**). In one specific embodiment, Form F of HCl salt of Compound (**1**)•3H_2O is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 7.1, 11.9, 19.2, and 12.4 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form F of HCl salt of Compound (**1**)•3H_2O is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 16.4, 21.8, and 23.9 (± 0.2) in an X-ray powder

diffraction pattern. In another specific embodiment, Form F of HCl salt of Compound (**1**)•$3H_2O$ is characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the following positions listed in Table 5. In yet another specific embodiment, Form F of HCl salt of Compound (**1**)•$3H_2O$ is characterized as having an XRPD pattern substantially the same as that shown in FIG. 3. The XRPD patterns are obtained at room temperature using Cu K alpha radiation. In yet another specific embodiment, the polymorphic Form F of HCl salt of Compound (**1**)•$3H_2O$ is characterized by peaks at 20.7, 27.4, 104.8, 142.5, 178.6 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, the polymorphic Form F of HCl salt of Compound (**1**)•$3H_2O$ is further characterized by one or more peaks corresponding to 154.3, 20.3, 132.3, and 21.1 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form F of HCl salt of Compound (**1**)•$3H_2O$ is characterized as having C^{13} SSNMR peaks listed in Table 6. In yet another specific embodiment, Form F of HCl salt of Compound (**1**)•$3H_2O$ is characterized as having a C^{13} SSNMR spectrum substantially the same as that shown in FIG. 4.

[0049] In one embodiment, the present invention is directed to polymorphic Form D of HCl salt of Compound (**1**). This form is a non-solvated form of HCl salt of Compound (**1**). In one specific embodiment, Form D of HCl salt of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 5.8, 17.1, and 19.5 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 5.3, 10.5, and 15.9 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the positions listed in Table 7. In yet another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized as having an XRPD pattern substantially the same as that shown in FIG. 5. The XRPD patterns are obtained at room temperature using Cu K alpha radiation. In yet another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized as having peaks at 29.4, 53.4, 113.3, 135.4, 177.8 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form D of HCl salt of Compound (**1**) is further characterized by one or more peaks corresponding to 22.9, 23.9, 26.0, and 31.6 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized as having C^{13} SSNMR peaks listed in Table 8. In yet another specific embodiment, Form D of HCl salt of Compound (**1**) is characterized as having a C^{13} SSNMR spectrum substantially the same as that shown in FIG. 6.

[0050] In one embodiment, the present invention is directed to polymorphic Form A of Compound (**1**). This form is a non-solvated, free base form of Compound (**1**). In one specific embodiment, Form A of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 15.5, 18.9, and 22.0 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form A of Compound (**1**) is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 11.8, 16.9, 25.5, and 9.1 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form A of Compound (**1**) is characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the positions listed in Table 10. In yet another specific embodiment, Form A of Compound (**1**) is characterized as having an XRPD pattern substantially the same as that shown in FIG. 7. The XRPD patterns are obtained at room temperature using Cu K alpha radiation. In yet another specific embodiment, Form A of Compound (**1**) is characterized as having peaks at 21.0, 28.5, 50.4, 120.8, 138.5, and 176.2 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form A of Compound (**1**) is characterized as having peaks at 30.1, 25.9, 22.8, and 25.0 (± 0.3 ppm) in a C^{13} SSNMR spectrum. In yet another specific embodiment, Form A of Compound (**1**) is characterized as having C^{13} SSNMR peaks listed in Table 11. In yet another specific embodiment, Form A of Compound (**1**) is characterized as having a C^{13} SSNMR spectrum substantially the same as that shown in FIG. 8.

[0051] In one embodiment, the present invention is directed to polymorphic Form A of tosylate salt of Compound (**1**). This form is a non-solvated form of tosylate salt of Compound (**1**). In one specific embodiment, Form A of tosylate salt of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 7.2, 9.3, 13.7, 14.3, 14.7, 16.9, 18.7, 26.3, and 26.9 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form A of tosylate salt of Compound (**1**) is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 6.0, 28.0, and 27.5 (± 0.2) in an X-ray powder diffraction pattern. In another specific embodiment, Form A of tosylate salt of Compound (**1**) is characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the following positions listed in Table 14. In yet another specific embodiment, Form A of tosylate salt of Compound (**1**) is characterized as having XRPD pattern substantially the same as that shown in FIG. 9. The XRPD patterns are obtained at room temperature using Cu K alpha radiation.

[0052] In another embodiment, the present invention is directed to methods of preparing Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O,

Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), and Form A of tosylate salt of Compound (**1**).

[0053] Form A of HCl salt of Compound (**1**)•1/2H_2O can be prepared by employing mixing (e.g., stirring) hydrogen chloride (HCl) with Compound (**1**). Compound (**1**) can be solvated, non-solvated, amorphous, or crystalline. A solution, slurry, or suspension of Compound (**1**) can be mixed with HCl in a solvent system that includes water and one or more organic solvents, wherein the solvent system has a water activity of equal to, or greater than, 0.05 and equal to, or less than, 0.85, i.e., 0.05 - 0.85. The term "water activity" (a_w) is used herein as known in the art and means a measure of the energy status of water in a solvent system. It is defined as the vapor pressure of a liquid divided by that of pure water at the same temperature. Specifically, it is defined as $a_w = \frac{p}{p_o}$, where p is the vapor pressure of water in the substance, and p_o is the vapor pressure of pure water at the same temperature, or as $a_w = l_w \times x_w$, where l_w is the activity coefficient of water and x_o is the mole fraction of water in the aqueous fraction. For example, pure water has a water activity value of 1.0. Water activity values can typically be obtained by either a capacitance hygrometer or a dew point hygrometer. Various types of water activity measuring instruments are also commercially available. Alternatively, water activity values of mixtures of two or more solvents can be calculated based on the amounts of the solvents and the known water activity values of the solvents.

[0054] An example of crystalline Compound (**1**) includes Form A of Compound (**1**). Examples of solvates of Compound (**1**) include solvates of 2-MeTHF, N,N-dimentylacetamide, N,N-dimethylformamide, methanol, xylene, acetone, 2-butanol, methyl acetate, 1-pentanol, 2-propanol, tetrahydrofuran, methyl tetrahydrofuran, dimethylacetamide N,N-dimethylformamide 1,4-dioxane, 1-pentanol, 2-methy-1-propanol, methylethyl ketone, 3-methyl-1-butanol, heptane, ethyl formate, 1-butanol, acetic acid, and ethylene glycol. In a specific embodiment, solvates of 2-MeTHF (e.g., Compound (**1**)•1(2-MeTHF)) are employed.

[0055] The solvent systems suitable for the preparation of Form A of HCl salt of Compound (**1**)•1/2H_2O can be comprised of a large variety of combinations of water and organic solvents where the water activity of the solvent systems is equal to, or greater than, 0.05 and equal to, or less than, 0.85 (0.05-0.85). In a specific embodiment, the value of the water activity is 0.4-0.6. Suitable organic solvents include Class II or Class III organic solvents listed in the International Conference on Harmonization Guidelines. Specific examples of suitable Class

II organic solvents include chlorobenzene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,2-dimethoxyethane, N,N-dimentylacetamide, N,N-dimethylformamide, 1,4-dioxane, 2-ethoxyethanol, formamide, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidone, nitromethane, pyridine, sulfolane, tetrahydrofuran (THF), tetralin, tolune, 1,1,2-trichloroethene and xylene. Specific examples of suitable Class III organic solvents include: acetic acid, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, methylisobutyl ketone, 2-methyl-1-propanol, ethyl acetate, ethyl ether, ethyl formate, pentane, 1-pentanol, 1-propanol, 2-propanol and propyl acetate. In one specific embodiment, the organic solvents of the solvent system are selected from the group consisting of chlorobenzene, cyclohexane, 1,2-dichloroethane, dichloromethane, 1,2-dimethoxyethane, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, nitromethane, tetralin, xylene, toluene, 1,1,2-trichloroethane, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, ethanol, ethyl acetate, ethyl ether, ethyl formate, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, 2-methy-1-propanol, pentane, 1-propanol, 1-pentanol, 2-propanol, propyl acetate, tetrahydrofuran, and methyl tetrahydrofuran. In another specific embodiment, the organic solvents of the solvent system are selected from the group consisting of 2-ethoxyethanol, ethyleneglycol, methanol, 2-methoxyethanol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, ethanol, 1-pentanol, 1-propanol, 2-propanol, methylbutyl ketone, acetone, methylethyl ketone, methylisobutyl ketone, butyl acetate, isobutyl acetate, isopropyl acetate, methyl acetate, ethyl acetate, propyl acetate, pyridine, toluene, and xylene. In yet another embodiment, the organic solvents are selected from the group consisting of acetone, n-propanol, isopropanol, iso-butylacetate, and acetic acid. In yet another embodiment, the organic solvents are selected from the group consisting of acetone and isopropanol. In yet another specific embodiment, the solvent system includes water an acetone. In yet another specific embodiment, the solvent system includes water an isopropanol.

[0056] The preparation of Form A of HCl salt of Compound (**1**)•1/2H_2O can be performed at any suitable temperature. Typically, it is performed at a temperature of 5 °C - 75 °C. In a specific embodiment, it is performed at a temperature of 15 °C - 75 °C. In another specific embodiment, it is performed at a temperature of 15 °C - 60 °C. In yet another specific embodiment, it is performed at a temperature of 15 °C - 35 °C. In yet another specific embodiment, the preparation is performed at 5 °C - 75 °C in a solvent system having a water

activity value of 0.4-0.6. In yet another specific embodiment, the preparation is performed at a temperature of 15 °C - 75 °C in a solvent system having a water activity value of 0.4 - 0.6. In yet another specific embodiment, the preparation is performed at a temperature of 15 °C - 60 °C in a solvent system having a water activity value of 0.4 - 0.6. In yet another specific embodiment, the preparation is performed at 15 °C - 35 °C in a solvent system having a water activity value of 0.4 - 0.6.

[0057] The hydrogen chloride (HCl) can be introduced as a solution or gas. One example, a suitable hydrogen chloride source is an aqueous solution of hydrogen chloride comprising 30-40 wt% (e.g., 34 wt% - 38 wt%) of HCl by weight of the aqueous solution.

[0058] Form F of HCl salt of Compound (**1**)•$3H_2O$ can be prepared by mixing HCl and Compound (**1**) in a solvent system that includes water or that includes water and one or more organic solvents, wherein the solvent system has a water activity of equal to, or greater than, 0.9 ($\geq$ 0.9). The mixture can be a solution, slurry, or suspension. Compound (**1**) can be solvated, non-solvated, amorphous, or crystalline. Alternatively, it can be prepared by stirring Form A of HCl salt of Compound (**1**)•$1/2H_2O$ in a solvent system that includes water or that includes water and one or more organic solvents, wherein the solvent system has a water activity of equal to, or greater than, 0.9. Typically, pure water has a water activity value of 1.0. Accordingly, a solvent system having a water activity of 0.9-1.0 can be suitable for the preparation of Form F of HCl salt of Compound (**1**)•$3H_2O$. In a specific embodiment, the mixing or stirring is performed at an ambient temperature (18 °C - 25 °C). In another specific embodiment, the mixing or stirring is performed at a temperature of 15 °C - 30 °C. In another specific embodiment, the mixing or stirring is performed at a temperature of 20 °C - 28 °C (e.g., 25 °C). Suitable organic solvents, including specific examples, for the formation of Form F of HCl salt of Compound (**1**)•$3H_2O$ are as described above for Form A of HCl salt of Compound (**1**)•$1/2H_2O$. In yet another specific embodiment, the solvent system includes water an acetone. In yet another specific embodiment, the solvent system includes water an isopropanol.

[0059] Form D of HCl salt of Compound (**1**) can be prepared by dehydrating Form A of HCl salt of Compound (**1**)•$1/2H_2O$. The dehydration can be done by any suitable means, such as heating or dry nitrogen purge, or both.

[0060] Form A of Compound (**1**) can be prepared by (a) stirring a mixture of amorphous Compound (**1**) or a solvate of Compound (**1**) (such as a 2-MeTHF solvate of Compound (**1**)) in a solvent system that includes water and ethanol. The mixture can be a solution or slurry. In a specific embodiment, the stirring step is performed at a temperature in a range of 18 °C

to 90 °C. In another specific embodiment, the stirring step (a) is performed at a refluxing temperature of the solvent system. In another specific embodiment, the solvent system includes 5 wt% to 15 wt% of water by weight of the solvent system. Examples of solvates of Compound (**1**) are as described above. In a specific embodiment, solvates of 2-MeTHF (e.g., Compound (**1**)•1(2-MeTHF)) are employed.

[0061] In another embodiment, the methods of preparing Form A of Compound (**1**) further comprises: (b) stirring amorphous form of Compound (**1**) in nitromethane to form crystalline seed of Form A of Compound (**1**); and (c) adding the crystalline seed of Form A of Compound (**1**) to the resulting mixture of the mixing step (a). In a specific embodiment, the methods further comprises: (b) stirring the amorphous form of Compound (**1**) in nitromethane to form crystalline seed of Form A of Compound (**1**); (c) cooling the resulting mixture of the mixing step (a) to a temperature in a range of 18 °C to 60 °C (e.g., 50 - 55 °C or 55 °C); and (d) adding the crystalline seed of Form A of Compound (**1**) to the resulting mixture step (c). In another specific embodiment, the methods further comprises adding water, prior to the addition of crystalline seed of Form A of Compound (**1**), to the resulting mixture that has gone through the refluxing step in an amount to have the resulting solvent system include water by 15 - 25 wt% after the addition of water. In yet another specific embodiment, the methods further comprises adding water to the mixture that includes crystalline seed of Form A of Compound (**1**) in an amount to have the resulting solvent system include water by 35 - 45 wt% after the addition of water. In yet another specific embodiment, the methods further comprises cooling the mixture that includes crystalline seed of Form A of Compound (**1**), after the addition of water, to a temperature of 0 °C -10 °C.

[0062] In one specific embodiment, the crystalline seed of Form A of Compound (**1**) can be prepared by 2-MeTHF solvate of Compound (**1**) in nitromethane. In one embodiment, the solvent system for the refluxing step includes 5-15 wt% (e.g., 8 wt%, 10 wt%, or 12 wt%) of water by weight of the solvent system.

[0063] Form A of tosylate salt of Compound (**1**) can be prepared by stirring a mixture of amorphous Compound (**1**) or a solvate of Compound (**1**) ((such as a 2-MeTHF solvate of Compound (**1**)), *p*-toluenesulfonic acid, and a solvent system that includes acetonitrile. In a specific embodiment, the mixing or stirring step is performed at an ambient temperature. In another specific embodiment, the mixing or stirring step is performed at a temperature of 15-30 °C. In another specific embodiment, the mixing or stirring step is performed at a temperature of 20-30 °C (e.g., 25 °C). Suitable examples of solvates of Compound (**1**),

including specific examples, are as described above for the preparation of Form A of Compound (**1**).

[0064] In yet another embodiment, the invention is directed to 2-MeTHF solvates of Compound (**1**). In one specific embodiment, the solvates include 0.5 - 1.5 equivalents of 2-MeTHF per Compound (**1**), such as 1 equivalent of 2-MeTHF per Compound (**1**). In one specific embodiment, the solvates include 1 equivalent of 2-MeTHF and characterized as having an XRPD pattern with characteristic peaks expressed in 2-theta ± 0.2 at the following positions at 8.4, 9.7, 16.7, 16.9, 17.4, 21.0, 22.3, and 25.7. In another specific embodiment, the solvates include 1 equivalent of 2-MeTHF and are characterized by having certain XRPD peaks listed in Table 12 or by having XRPD patterns as shown in FIG. 10.

[0065] In yet another embodiment, the invention encompasses amorphous forms of Compound (**1**) and pharmaceutically acceptable salts thereof, such as amorphous HCl salt of Compound (**1**) and amorphous Compound (**1**). In yet another embodiment, the invention also encompasses Form B of Compound (**1**) hydrate. Form B of Compound (**1**) hydrate is isomorphic with Form A of Compound (1), showing the same XRPD peaks as those for Form A of Compound (1), but formed in the presence of water, for example, in a system having a water activity greater than 0.6, such as 0.6 - 1.0, at ambient temperature.

[0066] The present invention encompasses the polymorphic forms of Compound (**1**) described above in isolated, pure form, or in a mixture as a solid composition when admixed with other materials, for example the other forms (i.e. amorphous form, Form A of Compound (**1**), etc.) of Compound (**I**) or any other materials.

[0067] In one aspect, the present invention provides polymorphic forms, such as Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), Form B of Compound (**1**) hydrate, and Form A of tosylate salt of Compound (**1**), in isolated solid form. In yet another aspect, the present invention provides amorphous form of Compound (**1**) and pharmaceutically acceptable salts thereof, such as amorphous HCl salt of Compound (**1**) and amorphous Compound (**1**), in isolated solid form.

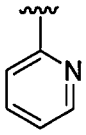
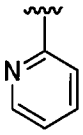
[0068] In a further aspect, the present invention provide polymorphic forms, such as Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), Form B of Compound (**1**) hydrate and Form A of tosylate salt of Compound (**1**), in pure form. The pure form means that the particular polymorphic form comprises over 95% (w/w), for example, over 98% (w/w), over 99% (w/w %), over 99.5% (w/w), or over 99.9% (w/w). In another further aspect there is

provided amorphous forms of Compound (**1**) or pharmaceutically acceptable salts thereof in pure form. The pure form means that the amorphous form is over 95% (w/w), for example, over 98% (w/w), over 99% (w/w %), over 99.5% (w/w), or over 99.9% (w/w).

[0069] More specifically, the present invention provides that each of the polymorphic forms in the form of a composition or a mixture of the polymorphic form with one or more other crystalline, solvate, amorphous, or other polymorphic forms or their combinations thereof. For example, in one embodiment, the composition comprises Form A of HCl salt of Compound (**1**)•1/2H_2O along with one or more other polymorphic forms of Compound (**1**), such as amorphous form, solvates, Form D of HCl salt of Compound (**1**), Form F of HCl salt of Compound (**1**)•3H_2O, Form A of Compound (**1**), and/or other forms or any combination thereof. Similarly, in another embodiment, the composition comprises Form F of HCl salt of Compound (**1**)•3H_2O along with one or more other polymorphic forms of Compound (**1**), such as amorphous form, solvates, Form A of HCl salt of Compound (**1**)•1/2H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), and/or other forms or their combinations thereof. Similarly, in another embodiment, the composition comprises Form D of HCl salt of Compound (**1**) along with one or more other polymorphic forms of Compound (**1**), such as amorphous form, solvates, Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form A of Compound (**1**), and/or other forms or their combinations thereof. In yet another embodiment, the composition comprises Form A of Compound (**1**) along with one or more other polymorphic forms of Compound (**1**), such as amorphous form, hydrates, solvates, and/or other forms or their combinations thereof. In yet another embodiment, the composition comprises Form A of tosylate salt of Compound (**1**) along with one or more other polymorphic forms of Compound (**1**), such as amorphous form, hydrates, solvates, and/or other forms or their combinations thereof. More specifically, the composition may comprise from trace amounts up to 100% of the specific polymorphic form or any amount, for example, in a range of 0.1% - 0.5%, 0.1% - 1%, 0.1% - 2%, 0.1% - 5%, 0.1% - 10%, 0.1% - 20%, 0.1% - 30%, 0.1% - 40%, or 0.1% - 50% by weight based on the total amount of Compound (**1**) in the composition. Alternatively, the composition may comprise at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99%, 99.5% or 99.9% by weight of specific polymorphic form based on the total amount of Compound (**1**) in the composition.

[0070] For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic

Chemistry", Thomas Sorrell, University Science Books, Sausolito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0071] Unless otherwise indicated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, cis-trans, conformational, and rotational) forms of the structure. For example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers are included in this invention, unless only one of the isomers is drawn specifically. As would be understood to one skilled in the art, a substituent can freely rotate around any rotatable bonds. For example, a substituent drawn as  also represents  .

[0072] Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, cis/trans, conformational, and rotational mixtures of the present compounds are within the scope of the invention.

[0073] Unless otherwise indicated, all tautomeric forms of the compounds of the invention are within the scope of the invention.

[0074] Additionally, unless otherwise indicated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools or probes in biological assays. Such compounds, especially deuterium (D) analogs, can also be therapeutically useful.

[0075] The compounds described herein are defined herein by their chemical structures and/or chemical names. Where a compound is referred to by both a chemical structure and a chemical name, and the chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity.

[0076] It will be appreciated by those skilled in the art that the compounds in accordance with the present invention can contain a chiral center. The compounds of formula may thus exist in the form of two different optical isomers (i.e. (+) or (-) enantiomers). All such enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. The single optical isomer or enantiomer can be obtained by method well known in the art, such as chiral HPLC, enzymatic resolution and chiral auxiliary.

[0077] In one embodiment, the compounds in accordance with the present invention are provided in the form of a single enantiomer at least 95%, at least 97% and at least 99% free of the corresponding enantiomer.

[0078] In a further embodiment, the compounds in accordance with the present invention are in the form of the (+) enantiomer at least 95% free of the corresponding (-) enantiomer.

[0079] In a further embodiment, the compounds in accordance with the present invention are in the form of the (+) enantiomer at least 97% free of the corresponding (-) enantiomer.

[0080] In a further embodiment, the compounds in accordance with the present invention are in the form of the (+) enantiomer at least 99% free of the corresponding (-) enantiomer.

[0081] In a further embodiment, the compounds in accordance with the present invention are in the form of the (-) enantiomer at least 95% free of the corresponding (+) enantiomer.

[0082] In a further embodiment, the compounds in accordance with the present invention are in the form of the (-) enantiomer at least 97% free of the corresponding (+) enantiomer.

[0083] In a further embodiment the compounds in accordance with the present invention are in the form of the (-) enantiomer at least 99% free of the corresponding (+) enantiomer.

[0084] II. USES OF COMPOUND **(1)** AND PHARMACEUTICALLY ACCEPTABLE SALTS THEREOF

[0085] One aspect of the present invention is generally related to the use of Compound **(1)** and its pharmaceutically acceptable salts, including the various solid forms (e.g., Form A of HCl salt of Compound **(1)**•1/2H_2O, Form F of HCl salt of Compound **(1)**•3H_2O, Form D of HCl salt of Compound **(1)**, Form A of Compound **(1)**, Form B of Compound **(1)** hydrate, and Form A of tosylate salt of Compound **(1)**) described above, for inhibiting the replication of influenza viruses in a biological sample or in a patient, for reducing the amount of influenza viruses (reducing viral titer) in a biological sample or in a patient, and for treating influenza in a patient. Hereinafter unless specifically indicated otherwise, Compound **(1)** and its pharmaceutically acceptable salts, including the various solid forms (e.g., Form A of HCl salt of Compound **(1)**•1/2H_2O, Form F of HCl salt of Compound **(1)**•3H_2O, Form D of HCl salt of Compound **(1)**, Form A of Compound **(1)**, Form B of Compound **(1)** hydrate, and Form A of tosylate salt of Compound **(1)**) described above, are referred to generally compounds.

[0086] In one embodiment, the present invention is generally related to the use of the compounds disclosed herein (e.g., in pharmaceutically acceptable compositions) for any of the uses specified above.

[0087] In yet another embodiment, the compounds disclosed herein can be used to reduce viral titre in a biological sample (e.g. an infected cell culture) or in humans (e.g. lung viral

titre in a patient).

[0088] The terms "influenza virus mediated condition", "influenza infection", or "Influenza", as used herein, are used interchangeably to mean the disease caused by an infection with an influenza virus.

[0089] Influenza is an infectious disease that affects birds and mammals caused by influenza viruses. Influenza viruses are RNA viruses of the family Orthomyxoviridae, which comprises five genera: Influenza virus A, Influenza virus B, Influenza virus C, ISA virus and Thogoto virus. Influenza virus A genus has one species, influenza A virus which can be subdivided into different serotypes based on the antibody response to these viruses: H1N1, H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3 and H10N7. Additional examples of influenza A virus include H3N8 and H7N9. Influenza virus B genus has one species, influenza B virus. Influenza B almost exclusively infects humans and is less common than influenza A. Influenza virus C genus has one species, Influenza virus C virus, which infects humans and pigs and can cause severe illness and local epidemics. However, Influenza virus C is less common than the other types and usually seems to cause mild disease in children.

[0090] In some embodiments of the invention, influenza or influenza viruses are associated with Influenza virus A or B. In some embodiments of the invention, influenza or influenza viruses are associated with Influenza virus A. In some specific embodiments of the invention, Influenza virus A is H1N1, H2N2, H3N2 or H5N1. In some specific embodiments of the invention, Influenza virus A is H1N1, H3N2, H3N8, H5N1, and H7N9. In some specific embodiments of the invention, Influenza virus A is H1N1, H3N2, H3N8, and H5N1.

[0091] In humans, common symptoms of influenza are chills, fever, pharyngitis, muscle pains, severe headache, coughing, weakness, and general discomfort. In more serious cases, influenza causes pneumonia, which can be fatal, particularly in young children and the elderly. Although it is often confused with the common cold, influenza is a much more severe disease and is caused by a different type of virus. Influenza can produce nausea and vomiting, especially in children, but these symptoms are more characteristic of the unrelated gastroenteritis, which is sometimes called "stomach flu" or "24-hour flu".

[0092] Symptoms of influenza can start quite suddenly one to two days after infection. Usually the first symptoms are chills or a chilly sensation, but fever is also common early in the infection, with body temperatures ranging from 38 °C to 39 °C (approximately 100 °F to 103 °F). Many people are so ill that they are confined to bed for several days, with aches and pains throughout their bodies, which are worse in their backs and legs. Symptoms of influenza may include: body aches, especially joints and throat, extreme coldness and fever,

fatigue, headache, irritated watering eyes, reddened eyes, skin (especially face), mouth, throat and nose, abdominal pain (in children with influenza B). Symptoms of influenza are non-specific, overlapping with many pathogens ("influenza-like illness"). Usually, laboratory data is needed in order to confirm the diagnosis.

[0093] The terms, "disease", "disorder", and "condition" may be used interchangeably here to refer to an influenza virus mediated medical or pathological condition.

[0094] As used herein, the terms "subject" and "patient" are used interchangeably. The terms "subject" and "patient" refer to an animal (e.g., a bird such as a chicken, quail or turkey, or a mammal), specifically a "mammal" including a non-primate (e.g., a cow, pig, horse, sheep, rabbit, guinea pig, rat, cat, dog, and mouse) and a primate (e.g., a monkey, chimpanzee and a human), and more specifically a human. In one embodiment, the subject is a non-human animal such as a farm animal (e.g., a horse, cow, pig or sheep), or a pet (e.g., a dog, cat, guinea pig or rabbit). In a preferred embodiment, the subject is a "human".

[0095] The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof.

[0096] As used herein, "multiplicity of infection" or "MOI" is the ratio of infectious agents (e.g. phage or virus) to infection targets (e.g. cell). For example, when referring to a group of cells inoculated with infectious virus particles, the multiplicity of infection or MOI is the ratio defined by the number of infectious virus particles deposited in a well divided by the number of target cells present in that well.

[0097] As used herein the term "inhibition of the replication of influenza viruses" includes both the reduction in the amount of virus replication (e.g. the reduction by at least 10 %) and the complete arrest of virus replication (i.e., 100% reduction in the amount of virus replication). In some embodiments, the replication of influenza viruses are inhibited by at least 50%, at least 65%, at least 75%, at least 85%, at least 90%, or at least 95%.

[0098] Influenza virus replication can be measured by any suitable method known in the art. For example, influenza viral titre in a biological sample (e.g. an infected cell culture) or in humans (e.g. lung viral titre in a patient) can be measured. More specifically, for cell based assays, in each case cells are cultured in vitro, virus is added to the culture in the presence or absence of a test agent, and after a suitable length of time a virus-dependent endpoint is evaluated. For typical assays, the Madin-Darby canine kidney cells (MDCK) and the standard tissue culture adapted influenza strain, A/Puerto Rico/8/34 can be used. A first type of cell assay that can be used in the invention depends on death of the infected target cells, a

process called cytopathic effect (CPE), where virus infection causes exhaustion of the cell resources and eventual lysis of the cell. In the first type of cell assay, a low fraction of cells in the wells of a microtiter plate are infected (typically 1/10 to 1/1000), the virus is allowed to go through several rounds of replication over 48-72 hours, then the amount of cell death is measured using a decrease in cellular ATP content compared to uninfected controls. A second type of cell assay that can be employed in the invention depends on the multiplication of virus-specific RNA molecules in the infected cells, with RNA levels being directly measured using the branched-chain DNA hybridization method (bDNA). In the second type of cell assay, a low number of cells are initially infected in wells of a microtiter plate, the virus is allowed to replicate in the infected cells and spread to additional rounds of cells, then the cells are lysed and viral RNA content is measured. This assay is stopped early, usually after 18-36 hours, while all the target cells are still viable. Viral RNA is quantitated by hybridization to specific oligonucleotide probes fixed to wells of an assay plate, then amplification of the signal by hybridization with additional probes linked to a reporter enzyme.

[0099] As used herein a "viral titer (or titre)" is a measure of virus concentration. Titer testing can employ serial dilution to obtain approximate quantitative information from an analytical procedure that inherently only evaluates as positive or negative. The titer corresponds to the highest dilution factor that still yields a positive reading; for example, positive readings in the first 8 serial twofold dilutions translate into a titer of 1:256. A specific example is viral titer. To determine the titer, several dilutions will be prepared, such as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}. The lowest concentration of virus that still infects cells is the viral titer.

[0100] As used herein, the terms "treat", "treatment" and "treating" refer to both therapeutic and prophylactic treatments. For example, therapeutic treatments includes the reduction or amelioration of the progression, severity and/or duration of influenza viruses mediated conditions, or the amelioration of one or more symptoms (specifically, one or more discernible symptoms) of influenza viruses mediated conditions, resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a compound or composition of the invention). In specific embodiments, the therapeutic treatment includes the amelioration of at least one measurable physical parameter of an influenza virus mediated condition. In other embodiments the therapeutic treatment includes the inhibition of the progression of an influenza virus mediated condition, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a

physical parameter, or both. In other embodiments the therapeutic treatment includes the reduction or stabilization of influenza viruses mediated infections. Antiviral drugs can be used in the community setting to treat people who already have influenza to reduce the severity of symptoms and reduce the number of days that they are sick.

[0101] The term "chemotherapy" refers to the use of medications, e.g. small molecule drugs (rather than "vaccines") for treating a disorder or disease.

[0102] The terms "prophylaxis" or "prophylactic use" and "prophylactic treatment" as used herein, refer to any medical or public health procedure whose purpose is to prevent, rather than treat or cure a disease. As used herein, the terms "prevent", "prevention" and "preventing" refer to the reduction in the risk of acquiring or developing a given condition, or the reduction or inhibition of the recurrence or said condition in a subject who is not ill, but who has been or may be near a person with the disease. The term "chemoprophylaxis" refers to the use of medications, e.g., small molecule drugs (rather than "vaccines") for the prevention of a disorder or disease.

[0103] As used herein, prophylactic use includes the use in situations in which an outbreak has been detected, to prevent contagion or spread of the infection in places where a lot of people that are at high risk of serious influenza complications live in close contact with each other (e.g. in a hospital ward, daycare center, prison, nursing home, or the like). It also includes the use among populations who require protection from the influenza but who either do not get protection after vaccination (e.g., due to weak immune system), or when the vaccine is unavailable to them, or when they cannot get the vaccine because of side effects. It also includes use during the two weeks following vaccination, since during that time the vaccine is still ineffective. Prophylactic use may also include treating a person who is not ill with the influenza or not considered at high risk for complications, in order to reduce the chances of getting infected with the influenza and passing it on to a high-risk person in close contact with him (for instance, healthcare workers, nursing home workers, or the like).

[0104] According to the US CDC, an influenza "outbreak" is defined as a sudden increase of acute febrile respiratory illness (AFRI) occurring within a 48 to 72 hour period, in a group of people who are in close proximity to each other (e.g. in the same area of an assisted living facility, in the same household, etc.) over the normal background rate or when any subject in the population being analyzed tests positive for influenza. One case of confirmed influenza by any testing method is considered an outbreak.

[0105] A "cluster" is defined as a group of three or more cases of AFRI occurring within a 48 to 72 hour period, in a group of people who are in close proximity to each other (e.g. in

the same area of an assisted living facility, in the same household, etc.).

[0106] As used herein, the "index case", "primary case" or "patient zero" is the initial patient in the population sample of an epidemiological investigation. When used in general to refer to such patients in epidemiological investigations, the term is not capitalized. When the term is used to refer to a specific person in place of that person's name within a report on a specific investigation, the term is capitalized as Patient Zero. Often, scientists search for the index case to determine how the disease spread and what reservoir holds the disease in between outbreaks. Note that the index case is the first patient that indicates the existence of an outbreak. Earlier cases may be found and are labeled primary, secondary, tertiary, and the like.

[0107] In one embodiment, the methods of the invention are a preventative or "pre-emptive" measure to a patient, specifically a human, having a predisposition to complications resulting from infection by an influenza virus. The term "pre-emptive" or "pre-emptively", as used herein, for example, in 'pre-emptive' use, is the prophylactic use in situations in which an "index case" or an "outbreak" has been confirmed, in order to prevent the spread of infection in the rest of the community or population group.

[0108] In another embodiment, the methods of the invention are applied as a "pre-emptive" measure to members of a community or population group, specifically humans, in order to prevent the spread of infection.

[0109] As used herein, an "effective amount" refers to an amount sufficient to elicit the desired biological response. In the present invention the desired biological response is to inhibit the replication of influenza virus, to reduce the amount of influenza viruses or to reduce or ameliorate the severity, duration, progression, or onset of a influenza virus infection, prevent the advancement of an influenza viruses infection, prevent the recurrence, development, onset or progression of a symptom associated with an influenza virus infection, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy used against influenza infections. The precise amount of compound administered to a subject will depend on the mode of administration, the type and severity of the infection and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. When co-administered with other antiviral agents, e.g., when co-administered with an anti-influenza medication, an "effective amount" of the second agent will depend on the type of drug used. Suitable dosages are known for approved agents and can be adjusted by the skilled artisan according to the condition of the subject, the type of

condition(s) being treated and the amount of a compound described herein being used. In cases where no amount is expressly noted, an effective amount should be assumed. For example, the compounds disclosed herein can be administered to a subject in a dosage range from between approximately 0.01 to 100 mg/kg body weight/day for therapeutic or prophylactic treatment.

[0110] Generally, dosage regimens can be selected in accordance with a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the renal and hepatic function of the subject; and the particular compound or salt thereof employed, the duration of the treatment; drugs used in combination or coincidental with the specific compound employed, and like factors well known in the medical arts. The skilled artisan can readily determine and prescribe the effective amount of the compounds described herein required to treat, to prevent, inhibit (fully or partially) or arrest the progress of the disease.

[0111] Dosages of the compounds described herein can range from 0.01 to 100 mg/kg body weight/day, 0.01 to 50 mg/kg body weight/day, 0.1 to 50 mg/kg body weight/day, or 1 to 25 mg/kg body weight/day. It is understood that the total amount per day can be administered in a single dose or can be administered in multiple dosing, such as twice a day (e.g., every 12 hours), three times a day (e.g., every 8 hours), or four times a day (e.g., every 6 hours).

[0112] In some embodiments, dosages of the compounds described herein (e.g., Compound (**1**) and its pharmaceutically acceptable salts thereof, including the various solid forms (e.g., Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), Form B of Compound (**1**) hydrate, and Form A of tosylate salt of Compound (**1**)) are in a range of 100 mg to 1,600 mg, such as 400 mg to 1,600 mg or 400 mg to 1,200 mg. Each dose can be taken once a day (QD), twice per day (e.g., every 12 hours (BID)), or three times per day (e.g., q8h (TID)). It is noted that any combinations of QD, BID, and TID can be employed, as desired, such as BID on day 1, followed by QD thereafter.

[0113] In some embodiments, dosages of the compounds described herein (e.g., Compound (**1**) and its pharmaceutically acceptable salts thereof, including the various solid forms (e.g., Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**)) are in a range of 100 mg to 1,600 mg, such as 400 mg

to 1,600 mg or 400 mg to 1,200 mg. Each dose can be taken once a day (QD), twice per day (e.g., every 12 hours (BID)), or three times per day (e.g., q8h (TID)). It is noted that any combinations of QD, BID, and TID can be employed, as desired, such as BID on day 1, followed by QD thereafter, or, when a loading dosage is employed on day 1, BID on day 2, followed by QD thereafter.

[0114] In one specific embodiment, dosages of the compounds described herein are 400 mg to 1,600 mg, 400 mg to 1,200 mg, or 600 mg to 1,200 mg once a day. In another specific embodiment, dosages of the compounds described herein are 400 mg to 1,600 mg, 400 mg to 1,200 mg, or 300 mg to 900 mg twice a day. In yet another specific embodiment, dosages of the compounds described herein are 400 mg to 1,000 mg once a day. In yet another specific embodiment, dosages of the compounds described herein are 600 mg to 1,000 mg once a day. In yet another specific embodiment, dosages of the compounds described herein are 600 mg to 800 mg once a day. In yet another specific embodiment, dosages of the compounds described herein are 400 mg to 800 mg twice a day (e.g., 400 mg to 800 mg every 12 hours). In yet another specific embodiment, dosages of the compounds described herein are 400 mg to 600 mg twice a day.

[0115] In some embodiments, a loading dosage regimen is employed. In one specific embodiment, a loading dose of 400 mg to 1,600 mg is employed on day 1 of treatment. In another specific embodiment, a loading dose of 600 mg to 1,600 mg is employed on day 1 of treatment. In another specific embodiment, a loading dose of 800 mg to 1,600 mg is employed on day 1 of treatment. In yet another specific embodiment, a loading dose of 900 mg to 1,600 mg is employed on day 1 of treatment. In yet another specific embodiment, a loading dose of 900 mg to 1,200 mg is employed on day 1 of treatment. In yet another specific embodiment, a loading dose of 900 mg is employed on day 1 of treatment. In yet another specific embodiment, a loading dose of 1,000 mg is employed on day 1 of treatment. In yet another specific embodiment, a loading dose of 1,200 mg is employed on day 1 of treatment.

[0116] In one specific embodiment, the dosage regimen of the compounds described herein employs a loading dosage of 600 mg to 1,600 mg on day 1 and with a regular dosage of 300 mg to 1,200 mg for the rest of the treatment duration. Each regular dose can be taken once a day, twice a day, or three times a day, or any combination thereof. In a further specific embodiment, a loading dosage of 900 mg to 1,600 mg, such as 900 mg, 1,200 mg, or 1,600 mg, is employed. In another further specific embodiment, a loading dosage of 900 mg to 1,200 mg, such as 900 mg or 1,200 mg, is employed. In yet another further specific

embodiment, a regular dosage of 400 mg to 1,200 mg, such as 400 mg, 600 mg, or 800 mg, is employed for the rest of the treatment duration. In yet another further specific embodiment, a regular dosage of 400 mg to 1,000 mg for the rest of the treatment duration. In yet another further specific embodiment, a regular dosage of 400 mg to 800 mg is employed for the rest of the treatment duration. In yet another further specific embodiment, a regular dosage of 300 mg to 900 mg twice a day is employed. In yet another further specific embodiment, a regular dosage of 600 mg to 1,200 mg once a day is employed. In yet another further specific embodiment, a regular dosage of 600 mg twice a day on day 2, followed by 600 mg once a day for the rest of the treatment duration.

[0117] For therapeutic treatment, the compounds described herein can be administered to a patient within, for example, 48 hours (or within 40 hours, or less than 2 days, or less than 1.5 days, or within 24 hours) of onset of symptoms (e.g., nasal congestion, sore throat, cough, aches, fatigue, headaches, and chills/sweats). Alternatively, for therapeutic treatment, the compounds described herein can be administered to a patient within, for example, 96 hours of onset of symptoms. The therapeutic treatment can last for any suitable duration, for example, for 3 days, 4 days, 5 days, 7 days, 10 days, 14 days, etc. For prophylactic treatment during a community outbreak, the compounds described herein can be administered to a patient within, for example, 2 days of onset of symptoms in the index case, and can be continued for any suitable duration, for example, for 7 days, 10 days, 14 days, 20 days, 28 days, 35 days, 42 days, etc., up to the entire flu season. A flu season is an annually-recurring time period characterized by the prevalence of outbreaks of influenza. Influenza activity can sometimes be predicted and even tracked geographically. While the beginning of major flu activity in each season varies by location, in any specific location these minor epidemics usually take 3-4 weeks to peak and another 3-4 weeks to significantly diminish. Typically, Centers for Disease Control (CDC) collects, compiles and analyzes information on influenza activity year round in the United States and produces a weekly report from October through mid-May.

[0118] In one embodiment, the therapeutic treatment lasts for 1 day to an entire flu season. In one specific embodiment, the therapeutic treatment lasts for 3 days to 14 days. In another specific embodiment, the therapeutic treatment lasts for 5 days to 14 days. In another specific embodiment, the therapeutic treatment lasts for 3 days to 10 days. In yet another specific embodiment, the therapeutic treatment lasts for 4 days to 10 days. In yet another specific embodiment, the therapeutic treatment lasts for 5 days to 10 days. In yet another specific embodiment, the therapeutic treatment lasts for 4 days to 7 days (e.g., 4 days, 5 days, 6 days, or 7 days). In yet another specific embodiment, the therapeutic treatment lasts for 5

days to 7 days (e.g., 5 days, 6 days, or 7 days). In one specific embodiment, the prophylactic treatment lasts up to the entire flu season.

[0119] In one specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days (e.g., 5 days to 14 days) with a loading dosage of 900 mg to 1,600 mg on day 1 and with a regular dosage of 300 mg to 1,200 mg for the rest of the treatment duration. In another specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days (e.g., 5 days to 14 days) with a loading dosage of 900 mg to 1,200 mg on day 1 and with a regular dosage of 400 mg to 1,000 mg for the rest of the treatment duration. In yet another specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days (e.g., 5 days to 14 days) with a loading dosage of 900 mg to 1,200 mg on day 1 and with a regular dosage of 400 mg to 800 mg for the rest of the treatment duration. In yet another specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days (e.g., 5 days to 14 days) with a loading dosage of 900 mg to 1,200 mg on day 1 and with a regular dosage of 400 mg to 800 mg for the rest of the treatment duration. Each dose can be taken once a day, twice a day, or three times a day, or any combination thereof.

[0120] In one specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days with a loading dosage of 900 mg to 1,600 mg on day 1 and with a regular dosage of 600 mg to 1,000 mg once a day for the rest of the treatment duration. In another specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days with a loading dosage of 900 mg to 1,200 mg on day 1 and with a regular dosage of 600 mg to 800 mg (e.g., 600 mg, 650 mg, 700 mg, 750 mg, or 800 mg) once a day for the rest of the treatment duration. In some embodiments, the treatment duration is for 4 days to 10 days, 5 days to 10 days, or 5 days to 7 days.

[0121] In one specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days with a loading dosage of 900 mg to 1,600 mg on day 1 and with a regular dosage of 400 mg to 800 mg twice a day for the rest of the treatment duration. In another specific embodiment, the compounds described herein are administered to a patient for 3 days to 14 days with a loading dosage of 900 mg to 1,200 mg on day 1 and with a regular dosage of 400 mg to 600 mg (e.g., 400 mg, 450 mg, 500 mg, 550 mg, or 600 mg) twice a day for the rest of the treatment duration. In some embodiments, the duration is for 4 days to 10 days, 5 days to 10 days, or 5 days to 7 days.

[0122] In one specific embodiment, the compounds described herein are administered to a patient for 4 days or 5 days with a loading dosage of 900 mg to 1,200 mg (e.g., 900 mg or

1,200 mg) on day 1 and with a regular dosage of 400 mg to 600 mg (e.g., 400 mg or 600 mg) twice a day for the rest of the treatment duration (e.g., days 2 through 4, or days 2 through 5). In another specific embodiment, the compounds described herein are administered to a patient for 4 days or 5 days with a loading dosage of 900 mg to 1,200 mg (e.g., 900 mg or 1,200 mg) on day 1 and with a regular dosage of 600 mg to 800 mg (e.g., 600 mg or 800 mg) once a day for the rest of the treatment duration.

[0123] Various types of administration methods can be employed in the invention, and are described in detail below under the section entitled "Administration Methods".

[0124] Various types of administration methods can be employed in the invention, and are described in detail below under the section entitled "Administration Methods".

[0125] III. COMBINATION THERAPY

[0126] An effective amount can be achieved in the method or pharmaceutical composition of the invention employing a compound of the invention (including a pharmaceutically acceptable salt or solvate (e.g., hydrate)) alone or in combination with an additional suitable therapeutic agent, for example, an antiviral agent or a vaccine. When "combination therapy" is employed, an effective amount can be achieved using a first amount of a compound of the invention and a second amount of an additional suitable therapeutic agent (e.g. an antiviral agent or vaccine).

[0127] In another embodiment of this invention, a compound of the invention and the additional therapeutic agent, are each administered in an effective amount (i.e., each in an amount which would be therapeutically effective if administered alone). In another embodiment, a compound of the invention and the additional therapeutic agent, are each administered in an amount which alone does not provide a therapeutic effect (a sub-therapeutic dose). In yet another embodiment, a compound of the invention can be administered in an effective amount, while the additional therapeutic agent is administered in a sub-therapeutic dose. In still another embodiment, a compound of the invention can be administered in a sub-therapeutic dose, while the additional therapeutic agent, for example, a suitable cancer-therapeutic agent is administered in an effective amount.

[0128] As used herein, the terms "in combination" or "co-administration" can be used interchangeably to refer to the use of more than one therapy (e.g., one or more prophylactic and/or therapeutic agents). The use of the terms does not restrict the order in which therapies (e.g., prophylactic and/or therapeutic agents) are administered to a subject.

[0129] Co-administration encompasses administration of the first and second amounts of the compounds of the coadministration in an essentially simultaneous manner, such as in a single

pharmaceutical composition, for example, capsule or tablet having a fixed ratio of first and second amounts, or in multiple, separate capsules or tablets for each. In addition, such coadministration also encompasses use of each compound in a sequential manner in either order.

[0130] In one embodiment, the present invention is directed to methods of combination therapy for inhibiting Influenza viruses replication in biological samples or patients, or for treating or preventing Influenza virus infections in patients using the compounds described herein. Accordingly, pharmaceutical compositions of the invention also include those comprising an inhibitor of Influenza virus replication of this invention in combination with an anti-viral compound exhibiting anti-Influenza virus activity.

[0131] Methods of use of the compounds described herein and compositions of the invention also include combination of chemotherapy with a compound or composition of the invention, or with a combination of a compound or composition of this invention with another anti-viral agent and vaccination with an Influenza vaccine.

[0132] When co-administration involves the separate administration of the first amount of a compound of the invention and a second amount of an additional therapeutic agent, the compounds are administered sufficiently close in time to have the desired therapeutic effect. For example, the period of time between each administration which can result in the desired therapeutic effect, can range from minutes to hours and can be determined taking into account the properties of each compound such as potency, solubility, bioavailability, plasma half-life and kinetic profile. For example, a compound of the invention and the second therapeutic agent can be administered in any order within 24 hours of each other, within 16 hours of each other, within 8 hours of each other, within 4 hours of each other, within 1 hour of each other or within 30 minutes of each other.

[0133] More, specifically, a first therapy (e.g., a prophylactic or therapeutic agent such as a compound of the invention) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (e.g., a prophylactic or therapeutic agent such as an anti-cancer agent) to a subject.

[0134] It is understood that the method of co-administration of a first amount of a compound

of the invention and a second amount of an additional therapeutic agent can result in an enhanced or synergistic therapeutic effect, wherein the combined effect is greater than the additive effect that would result from separate administration of the first amount of a compound of the invention and the second amount of an additional therapeutic agent.

[0135] As used herein, the term "synergistic" refers to a combination of a compound of the invention and another therapy (e.g., a prophylactic or therapeutic agent), which is more effective than the additive effects of the therapies. A synergistic effect of a combination of therapies (e.g., a combination of prophylactic or therapeutic agents) can permit the use of lower dosages of one or more of the therapies and/or less frequent administration of said therapies to a subject. The ability to utilize lower dosages of a therapy (e.g., a prophylactic or therapeutic agent) and/or to administer said therapy less frequently can reduce the toxicity associated with the administration of said therapy to a subject without reducing the efficacy of said therapy in the prevention, management or treatment of a disorder. In addition, a synergistic effect can result in improved efficacy of agents in the prevention, management or treatment of a disorder. Finally, a synergistic effect of a combination of therapies (e.g., a combination of prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

[0136] When the combination therapy using the compounds of the present invention is in combination with an Influenza vaccine, both therapeutic agents can be administered so that the period of time between each administration can be longer (e.g. days, weeks, or months).

[0137] The presence of a synergistic effect can be determined using suitable methods for assessing drug interaction. Suitable methods include, for example, the Sigmoid-Emax equation (Holford, N.H.G. and Scheiner, L.B., Clin. Pharmacokinet. 6: 429-453 (1981)), the equation of Loewe additivity (Loewe, S. and Muischnek, H., Arch. Exp. Pathol Pharmacol. 114: 313-326 (1926)) and the median-effect equation (Chou, T.C. and Talalay, P., Adv. Enzyme Regul. 22: 27-55 (1984)). Each equation referred to above can be applied with experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

[0138] Specific examples that can be co-administered with a compound described herein include neuraminidase inhibitors, such as oseltamivir (Tamiflu®) and Zanamivir (Rlenza®), viral ion channel (M2 protein) blockers, such as amantadine (Symmetrel®) and rimantadine (Flumadine®), and antiviral drugs described in WO 2003/015798, including T-705 under development by Toyama Chemical of Japan. (See also Ruruta et al., Antiviral Research, 82:

95-102 (2009), "T-705 (flavipiravir) and related compounds: Novel broad-spectrum inhibitors of RNA viral infections"). In some embodiments, the compounds described herein can be co-administered with a traditional influenza vaccine.

[0139] In some embodiments, the compounds described herein (e.g., Compound (**1**) and its pharmaceutically acceptable salts thereof, such as Form A of HCl salt of Compound (**1**)•1/2H_2O, Form F of HCl salt of Compound (**1**)•3H_2O, Form D of HCl salt of Compound (**1**), Form A of Compound (**1**), Form B of Compound (**1**) hydrate, and Form A of tosylate salt of Compound (**1**)) can be co-administered with zanamivir. In some embodiments, the compounds described herein can be co-administered with flavipiravir (T-705). In some embodiments, the compounds described herein can be co-administered with oseltamivir. In some embodiments, the compounds described herein can be co-administered with amantadine or rimantadine. Oseltamivir can be administered in a dosage regimen specified in its label. In some specific embodiments, it is administered 75 mg twice a day, or 150 mg once a day.

[0140] *Pharmaceutical Compositions*

[0141] The compounds described herein can be formulated into pharmaceutical compositions that further comprise a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle. In one embodiment, the present invention relates to a pharmaceutical composition comprising a compound of the invention described above, and a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle. In one embodiment, the present invention is a pharmaceutical composition comprising an effective amount of a compound of the present invention or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle. Pharmaceutically acceptable carriers include, for example, pharmaceutical diluents, excipients or carriers suitably selected with respect to the intended form of administration, and consistent with conventional pharmaceutical practices.

[0142] An "effective amount" includes a "therapeutically effective amount" and a "prophylactically effective amount". The term "therapeutically effective amount" refers to an amount effective in treating and/or ameliorating an influenza virus infection in a patient infected with influenza. The term "prophylactically effective amount" refers to an amount effective in preventing and/or substantially lessening the chances or the size of influenza virus infection outbreak. Specific examples of effective amounts are described above in the section entitled Uses of Disclosed Compounds.

[0143] A pharmaceutically acceptable carrier may contain inert ingredients which do not unduly inhibit the biological activity of the compounds. The pharmaceutically acceptable carriers should be biocompatible, e.g., non-toxic, non-inflammatory, non-immunogenic or

devoid of other undesired reactions or side-effects upon the administration to a subject. Standard pharmaceutical formulation techniques can be employed.

[0144] The pharmaceutically acceptable carrier, adjuvant, or vehicle, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds described herein, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this invention. As used herein, the phrase "side effects" encompasses unwanted and adverse effects of a therapy (e.g., a prophylactic or therapeutic agent). Side effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a therapy (e.g., prophylactic or therapeutic agent) might be harmful or uncomfortable or risky. Side effects include, but are not limited to fever, chills, lethargy, gastrointestinal toxicities (including gastric and intestinal ulcerations and erosions), nausea, vomiting, neurotoxicities, nephrotoxicities, renal toxicities (including such conditions as papillary necrosis and chronic interstitial nephritis), hepatic toxicities (including elevated serum liver enzyme levels), myelotoxicities (including leukopenia, myelosuppression, thrombocytopenia and anemia), dry mouth, metallic taste, prolongation of gestation, weakness, somnolence, pain (including muscle pain, bone pain and headache), hair loss, asthenia, dizziness, extra-pyramidal symptoms, akathisia, cardiovascular disturbances and sexual dysfunction.

[0145] Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffer substances (such as twin 80, phosphates, glycine, sorbic acid, or potassium sorbate), partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes (such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, or zinc salts), colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, methylcellulose, hydroxypropyl methylcellulose, wool fat, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato

starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol or polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[0146] IV. ADMINISTRATION METHODS

[0147] The compounds and pharmaceutically acceptable compositions described above can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated.

[0148] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0149] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or

suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[0150] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0151] In order to prolong the effect of a compound described herein, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

[0152] Compositions for rectal or vaginal administration are specifically suppositories which can be prepared by mixing the compounds described herein with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0153] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for

example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0154] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

[0155] The active compounds can also be in microencapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[0156] Dosage forms for topical or transdermal administration of a compound described herein include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use

of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[0157] The compositions described herein may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes, but is not limited to, subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Specifically, the compositions are administered orally, intraperitoneally or intravenously.

[0158] Sterile injectable forms of the compositions described herein may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0159] The pharmaceutical compositions described herein may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include, but are not limited to, lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents

include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0160] Alternatively, the pharmaceutical compositions described herein may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

[0161] The pharmaceutical compositions described herein may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0162] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[0163] For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2 octyldodecanol, benzyl alcohol and water.

[0164] For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, specifically, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

[0165] The pharmaceutical compositions may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability,

fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0166] The compounds for use in the methods of the invention can be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for subjects undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form can be for a single daily dose or one of multiple daily doses (e.g., 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form can be the same or different for each dose.

[0167] V. EXAMPLES

[0168] Example 1: General Methods of XRPD, C^{13} Solid State NMR, DSC Measurements

[0169] *Thermogravimetric analysis (TGA)*

[0170] Thermogravimetric analysis (TGA) was performed on the TA Instruments TGA model Q500 Asset Tag V014840. The solid sample was placed in a platinum sample pan and heated at 10 °C/min to 300 °C from room temperature.

[0171] *DSC Measurements*

[0172] Differential scanning calorimetry (DSC) was conducted on a TA Instruments DSC Q200 Asset Tag V015553. Approximately 1-2 mg of solid sample was placed in an aluminum hermetic DSC pan with a crimped lid with a pinhole. The sample cell was generally heated under nitrogen purge.

[0173] *SSNMR experimental*

[0174] Solid state nuclear magnetic spectroscopy (SSNMR) spectra were acquired on the Bruker-Biospin 400 MHz Advance III wide-bore spectrometer equipped with Bruker-Biospin 4mm HFX probe. Samples were packed into 4mm ZrO_2 rotors (approximately 70 mg or less, depending on sample availability). Magic angle spinning (MAS) speed of typically 12.5 kHz was applied. The temperature of the probe head was set to 275K to minimize the effect of frictional heating during spinning. The proton relaxation time was measured using ^1H MAS T_1 saturation recovery relaxation experiment in order to set up proper recycle delay of the ^{13}C cross-polarization (CP) MAS experiment. The recycle delay of ^{13}C CPMAS experiment was adjusted to be at least 1.2 times longer than the measured ^1H T_1 relaxation time in order to maximize the carbon spectrum signal-to-noise ratio. The CP contact time of ^{13}C CPMAS experiment was set to 2 ms. A CP proton pulse with linear ramp (from 50% to 100%) was employed. The Hartmann-Hahn match was optimized on external reference sample (glycine). Fluorine spectra were acquired using proton decoupled MAS setup with recycled

delay set to approximately 5 times of the measured ^{19}F T_1 relaxation time. The fluorine relaxation time was measured using proton decoupled ^{19}F MAS T_1 saturation recovery relaxation experiment. Both carbon and fluorine spectra were acquired with SPINAL 64 decoupling was used with the field strength of approximately 100 kHz. The chemical shift was referenced against external standard of adamantane with its upfield resonance set to 29.5 ppm.

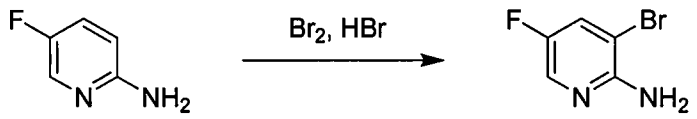
[0175] *Bruker D8 Discover XRPD Experimental Details*

[0176] The X-ray powder diffraction (XRPD) patterns were acquired at room temperature in reflection mode using a Bruker D8 Discover diffractometer (Asset Tag V012842) equipped with a sealed tube source and a Hi-Star area detector (Bruker AXS, Madison, WI). The X-Ray generator was operating at a voltage of 40 kV and a current of 35 mA. The powder sample was placed in an aluminum holder. Two frames were registered with an exposure time of 120 s each. The data was subsequently integrated over the range of 4.5°-39° 2Θ with a step size of 0.02° and merged into one continuous pattern.

[0177] **Example 2: Preparation of Compound (1) and 2-MeTHF solvate of Compound (1)**

[0178] Compound (**1**) can be prepared as described in WO 2010/148197. For example, an amorphous free base Compound (**1**) was prepared according to WO 2010/148197, followed by usual chiral separation and purification: SCF chiral chromatography with a modifier that included Et_2NH (which generated Et_2NH salt of Compound (**1**)) and then ion-exchange resin treatment. Its XRPD data is shown in FIG. 11. Alternatively, Compound (**1**) can be made by the following procedures as a 2-MeTHF solvate:

[0179] *Preparation of Compound 2a (2- Amino-3-bromo-5-fluoropyridine)*



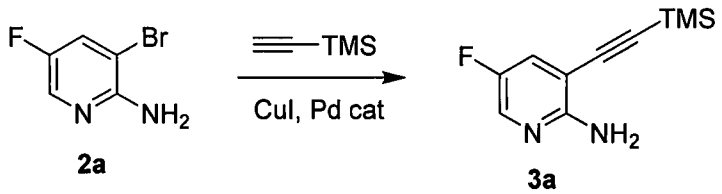
1a **2a**

[0180] To a slurry of 2-amino-5-fluoropyridine (6 kg, 53.6 mol) in water (24 L) at 14 °C was added over 10 minutes 48% hydrobromic acid (18.5 kg, 110 mol). The reaction was exothermic and the temperature went up to 24 °C. The mixture was re-cooled to 12 °C then bromine (9 kg, 56.3 mol) was added in nine portions over 50 minutes (exothermic, kept at 20 °C). The mixture was stirred at 22 °C overnight, and monitored by ^{1}HNMR of a quenched aliquot (quenched 5 drops in to mix of 1 ml 20% K_2CO_3, 0.3 ml 10% $Na_2S_2O_3$ and 0.7 ml DCM. Organic layer evaporated and assayed). The mixture was cooled to 10 °C then

quenched by addition of sodium bisulfite (560 g, 5.4 mol) in water (2 L), and further cooled to 0 °C. This mixture was added to a cold (-4 °C) mixture of DCM (18 L) and 5.4M sodium hydroxide (35 L, 189 mol). The bottom ~35 L was filtered through a pad of Celite and then the phase break was made. The aqueous layer was re-extracted with DCM (10 L). The organics were filtered through a pad of 3 kg magnesol, washing with DCM (8 L). The filtrate was evaporated, triturated with hexane and filtered.

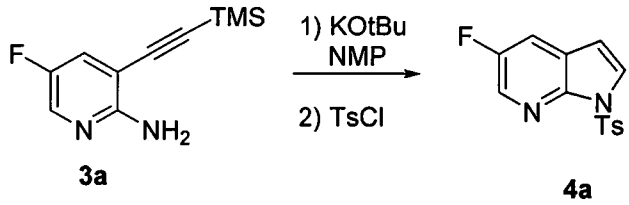
[0181] Despite the in-process assay indicating 97% completion, this initial product from all four runs typically contained ~10% SM. These were combined and triturated in hexane (2 L per kg material) at 50 °C, then cooled to 15 °C and filtered to afford Compound **2a** (30.0 kg, ~95% purity, 149 mol, 67%). Mother liquors from the initial trituration and the re-purification were chromatographed (20 kg silica, eluent 25-50% EtOAc in hexane) to afford additional Compound **2a** (4.7 kg, ~99% purity, 24.4 mol, 11%).

[0182] *Preparation of Compound 3a*



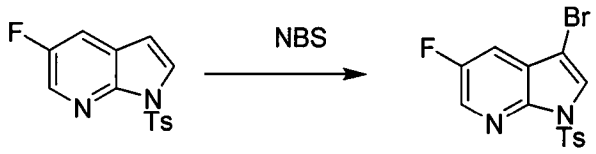
[0183] To an inert 400-L reactor was charged **2a** (27.5 kg, 96% purity, 138 mol), $Pd(PPh_3)_4$ (1044 g, 0.90 mol) and CuI (165 g, 0.87 mol), followed by toluene (90 kg). The mixture was de-oxygenated with three vacuum-nitrogen cycles, then triethylamine (19.0 kg, 188 mol) was added. The mixture was de-oxygenated with one more vacuum-nitrogen cycle, then TMS-acetylene (16.5 kg, 168 mol) was added. The mixture was heated to 48 °C for 23 hours (the initial exotherm took the temperature to 53° C maximum), then cooled to 18 °C. The slurry was filtered through a pad of Celite and washed with toluene (80 kg). The filtrate was washed with 12% Na_2HPO_4 (75 L), then filtered through a pad of silica (25 kg), washing with 1:1 hexane:MTBE (120 L). This filtrate was evaporated to a brown oil and then dissolved in NMP for the next step. Weight of a solution of Compound **3a** - 58 kg, ~50 wt%, 138 mol, 100%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.90 (s, 1H); 7.33-7.27 (m, 1H); 4.92 (s, NH_2), 0.28 (s, 9H) ppm.

[0184] *Preparation of Compound 4a*



[0185] To an inert 400-L reactor was charged potassium tert-butoxide (17.5 kg, 156 mol) and NMP (45 kg). The mixture was heated to 54 °C then a solution of Compound **3a** (29 kg, 138 mol) in NMP (38 kg) was added over 2.75 hours and rinsed in with NMP (6 kg) (exothermic, maintained at 70 °C - 77 °C). The reaction was stirred at 74 °C for 2 hours then cooled to 30 °C and a solution of tosyl chloride (28.5 kg, 150 mol) in NMP (30 kg) added over 1.5 hours and rinsed in with NMP (4 kg). The reaction was exothermic and maintained at 30 °C - 43 °C. The reaction was stirred for 1 hour while cooling to 20 °C then water (220 L) was added over 35 minutes (exothermic, maintained at 18 °C - 23 °C). The mixture was stirred at 20 °C for 30 minutes then filtered and washed with water (100 L). The solids were dissolved off the filter with DCM (250 kg), separated from residual water and the organics filtered through a pad of magnesol (15 kg, top) and silica (15 kg, bottom), washing with extra DCM (280 kg). The filtrate was concentrated to a thick slurry (~50 L volume) then MTBE (30 kg) was added while continuing the distillation at constant volume (final distillate temperature of 51 °C). Additional MTBE (10 kg) was added and the slurry cooled to 15 °C, filtered and washed with MTBE (40 L) to afford Compound **4a** (19.13 kg, 95% purity, 62.6 mol, 45%). Partial concentration of the filtrate afforded a second crop (2.55 kg, 91% purity, 8.0 mol, 6%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.28-8.27 (m, 1H); 8.06-8.02 (m, 2H); 7.77 (d, *J*= 4.0 Hz, 1H); 7.54-7.50 (m, 1H); 7.28-7.26 (m, 2H); 6.56 (d, *J*= 4.0 Hz, 1H); 2.37 (s, 3H) ppm.

[0186] *Preparation of Compound 5a*

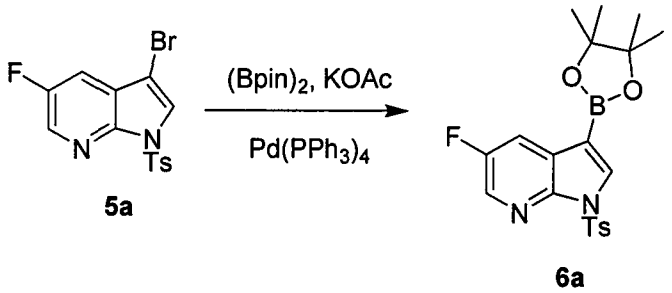


4a **5a**

[0187] To a slurry of N-bromosuccinimide (14.16 kg, 79.6 mol) in DCM (30 kg) at 15 °C was charged a solution of Compound **4a** (19.13 kg, 95% purity, and 2.86 kg, 91% purity, 71.6 mol) in DCM (115 kg), rinsing in with DCM (20 kg). The mixture was stirred at 25 °C for 18 hours, and then cooled to 9 °C and quenched by addition of a solution of sodium thiosulfate (400 g) and 50% sodium hydroxide (9.1 kg) in water (130 L). The mixture was warmed to 20 °C and the layers were separated and the organics were washed with 12% brine (40 L). The aqueous layers were sequentially re-extracted with DCM (4 × 50 kg). The organics were combined and 40 L distilled to azeotrope water, then the solution was filtered through a pad of silica (15 kg, bottom) and magensol (15 kg, top), washing with DCM (180 kg). The filtrate was concentrated to a thick slurry (~32 L volume) then hexane (15 kg)

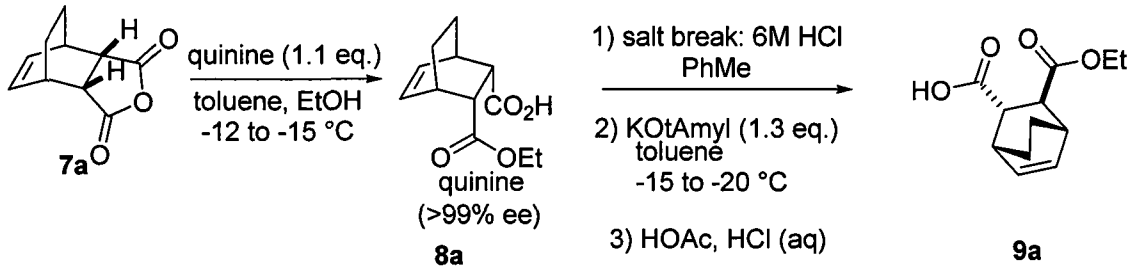
was added. Additional hexane (15 kg) was added while continuing the distillation at constant volume (final distillate temperature 52 °C). The slurry was cooled to 16 °C, filtered and washed with hexane (25 kg) to afford Compound **5a** (25.6 kg, 69.3 mol, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.34-8.33 (m, 1H); 8.07 (d, *J*= 8.2Hz, 2H); 7.85 (s, 1H); 7.52-7.49 (m, 1H); 7.32-7.28 (m, 2H); 2.40 (s, 3H) ppm.

[0188] *Preparation of Compound 6a: BEFTAI Reaction*



[0189] To an inert 400-L reactor was charged Compound **5a** (25.6 kg, 69.3 mol), bis(pinacolato)diboron (19 kg, 74.8 mol), potassium acetate (19 kg, 194 mol), palladium acetate (156 g, 0.69 mol) and triphenylphosphine (564 g, 2.15 mol), followed by dioxane (172 kg), that had been separately de-oxygenated using vacuum-nitrogen cycles (× 3). The mixture was stirred and de-oxygenated using vacuum-nitrogen cycles (× 2), then heated to 100 °C for 15 hours. The mixture was cooled to 35 °C then filtered, washing with 30 °C THF (75 kg). The filtrate was evaporated and the residue dissolved in DCM (~90 L). The solution was stirred with 1 kg carbon and 2 kg magnesol for 45 minutes then filtered through a pad of silica (22 kg, bottom) and magensol (10 kg, top), washing with DCM (160 kg). The filtrate was concentrated to a thick slurry (~40 L volume) then triturated at 35 °C and hexane (26 kg) was added. The slurry was cooled to 20 °C, filtered and washed with a mix of DCM (5.3 kg) and hexane (15 kg), then hexane (15 kg) and dried under nitrogen on the filter to afford Compound **6a** (23.31 kg, 56.0 mol, 81%) as a white solid. ^{1}H-NMR consistent with desired product, HPLC 99.5%, palladium assay 2 ppm. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.25 (s, 1H); 8.18 (s, 1H); 8.09-8.02 (m, 2H); 7.91-7.83 (m, 1H); 7.30-7.23 (m, 2H); 2.39 (s, 3H); 1.38 (s, 12H) ppm.

[0190] *Preparation of Compounds 8a and 9a*



[0191] *Compound 8a*: Anhydride **7a** (24.6 kgs, Apex) and quinine (49.2 kgs, Buchler) were added to a reactor followed by the addition of anhydrous PhMe (795.1 kgs). The reactor was then cooled to -16 °C and EtOH (anhydrous, 41.4 kgs) was added at such a rate to maintain the internal reactor temperature at less than -12 °C. The maximum reaction temp recorded for this experiment was -16 °C. The reaction mixture was then stirred for 16 h at -16 °C. A sample was removed and filtered. The solid was dried and evaluated by ^{1}H-NMR which showed that no anhydride remained. The contents of the reactor were filtered. The reactor and subsequent wet cake were washed with PhMe (anhydrous, 20 kgs). The resulting solid was placed in a tray dryer at less than 45 °C with a N_2 sweep for at least 48 h. In this experiment, the actual temperature was 44 °C and the vacuum was -30 inHg. Material was sampled after 2.5 d drying and showed 3% PhMe by NMR. After an additional 8 hrs, the amount of PhMe analyzed showed the same 3% PhMe present and the drying was stopped. The weight of the white solid was 57.7 kgs, 76% yield. ^{1}H-NMR showed consistent with structure and Chiral SFC analysis showed material >99% ee.

[0192] *Compound 9a*: The reactor was charged with quinine salt **8a** (57.7 kgs) and PhMe (250.5 kgs, Aldrich ACS grade, >99.5%) and the agitator was started. The contents were cooled to less than 15 °C and was treated with 6N HCl (18 kgs H_2O were treated with 21.4 kgs of conc. HCl) while keeping the temperature less than 25 °C. The mixture was stirred for 40 min and visually inspected to verify that no solids were present. Stirring was stopped and the phases were allowed to settle and phases were separated. The aqueous phases were extracted again with PhMe (160 kgs); the amount typically used was much less, calc. 43 kgs. However, for efficient stirring due to minimal volume, additional PhMe was added. The organic phases were combined. Sample the organic phase and run HPLC analysis to insure product is present; for information only test.

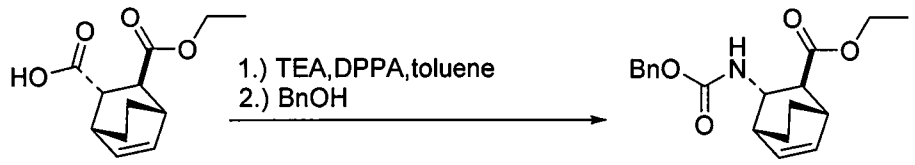
[0193] To the organic phases were cooled to less than 5 °C (e.g., 0 °C to 5 °C) and was added sodium sulfate (anhydrous, 53.1 kgs) with agitation for 8 hrs (in this instance 12 hrs). The contents of the reactor containing the organic phase were passed through a filter containing sodium sulfate (31 kgs, anhydrous) and into a cleaned and dried reactor. The reactor was rinsed with PhMe (57.4 kgs), passed through the filter into reactor 201. The agitator was started and an additional amount of PhMe (44 kgs) was added and the reaction mixture cooled to -20 °C. At that temperature PhMe solution of potassium tert-pentoxide was added over 2 h while keeping the temperature between -15 and -22 °C. The reaction mixture was held at approximately -20 °C for an additional 30 min before being sampled.

Sampling occurred by removing an aliquot with immediate quenching into 6N HCl. The target ratio here is 96:4 (trans:cis).

[0194] Having achieved the target ratio, the reactor was charged with acetic acid (2.8 kgs) over 6 min. The temperature stayed at -20 °C. The temperature was then adjusted to -5 °C and aqueous 2N HCl (65.7 kgs water treated with 15.4 kgs of conc. HCl) was added. The contents were warmed to 5 °C +/- 5 °C, agitated for 45 min before warming to 20 °C +/- 5 °C with stirring for 15 min. The agitator was stopped and the phases allowed to settle. The aqueous layer was removed (temporary hold). The organic phase was washed with water (48 kgs, potable), agitated for 15 min and phases allowed to settle (at least 15 min) and the aqueous layer was removed and added to the aqueous layer. 1/3 of a buffer solution (about 50 L) that was prepared (7.9 kgs NaH_2PO_4, 1.3 kgs of Na_2HPO_4 and 143.6 kgs water) was added to the organic phase and stirred for at least 15 min. Agitation was stopped and phases were allowed to separate for at least 15 min. The lower layer was discarded. Another portion of the buffered solution (about 50 L) was used to wash the organic layer as previously described. The wash was done a third time as described above.

[0195] Vacuum distillation of the PhMe phase (150 L) was started at 42 °C/-13.9 psig and distilled to an oil of approximately 20 L volume. After substantial reduction in volume the mixture was transferred to a smaller vessel to complete the distillation. Heptanes (13.7 kgs) was added and the mixture warmed to 40 +/- 5 °C for 30 min then the contents were cooled to 0 °C to 5 °C over 1.5 h. The solids were filtered and the reactor washed with approximately 14 kgs of cooled (0-5 °C) heptanes. The solids were allowed to dry under vacuum before placing in the oven at less than 40 °C under house vacuum (-28 psig) until LOD is <1%. 15.3 kgs, 64%, 96% HPLC purity. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.45 (br. s, 1H), 6.41 (t, J = 7.2 Hz, 1H), 6.25 (t, J = 7.2 Hz, 1H), 4.18 (m, 2H), 3.27 (m, 1H), 3.03 (m, 1H), 2.95 (m, 1H), 2.77 (m, 1H), 1.68 (m, 1H), 1.49 (m, 1H), 1.25 (t, J = 7.2Hz), 1.12 (m, 1H).

[0196] *Preparation of Compound 10a*



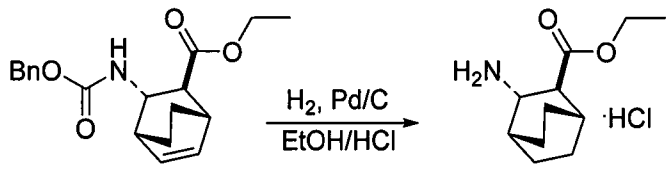
9a **10a**

[0197] A three neck flask equipped with a mechanical stirrer, temperature probe, reflux condenser, addition funnel and nitrogen inlet was charged with Compound **9a** (145.0 g, 1 equiv.) and anhydrous toluene (Aldrich, cat# 244511) (1408 g, 1655 ml) under an atmosphere of nitrogen. Then triethylamine (Aldrich, cat# 471283) (140 g, 193 ml,

2.14 equiv.) was added in portions over 5 minutes to the stirred solution during which an exotherm to a maximum temperature of 27 °C was observed. Data acquisition by ReactIR was started. The reaction mixture was then heated to 95 °C over 70 minutes. Then diphenyl phosphoryl azide (Aldrich, cat# 178756) (176.2 g; 138.0 ml, 0.99 equiv.) was added by addition funnel in portions over a total time of 2.25 hours.

[0198] Following completion of the addition of diphenyl phosphoryl azide (addition funnel rinsed with a small amount of toluene), the resulting mixture was heated at 96 °C for an additional 50 minutes. A sample of the reaction mixture diluted in toluene was analyzed by GC/MS which indicated consumption of diphenyl phosphoryl azide. Then benzyl alcohol (Aldrich, cat# 108006) (69.9 g, 67.0 ml, 1.0 equiv.) was added by addition funnel over 5-10 minutes. The resulting mixture was then heated at 97 °C overnight (for approximately 19 hours). A sample of the reaction mixture diluted in toluene by GC/MS indicated formation of product (m/e =330). The reaction mixture was then cooled to 21 °C after which water (870 g, 870 ml) was added in portions (observed slight exotherm to maximum temperature of 22 °C). The reaction mixture was first quenched by addition of 500 g of water and mechanically stirred for 10 minutes. The mixture was then transferred to the separatory funnel containing the remaining 370 g of water and then manually agitated. After agitation and phase separation, the organic and aqueous layers were separated (aqueous cut at pH of ~10). The organic layer was then washed with an additional portion of water (870 g; 1 × 870 ml). The organic and aqueous layers were separated (aqueous cut at pH of ~10). The collected organic phase was then concentrated to dryness under reduced pressure (water bath at 45 °C to 50 °C) affording 215 g of crude Compound **10a** (approximate volume of 190 ml). The ^{1}H NMR and GC/MS conformed to compound **10a** (with residual toluene and benzyl alcohol).

[0199] *Preparation of Compound 11a*



10a **11a**

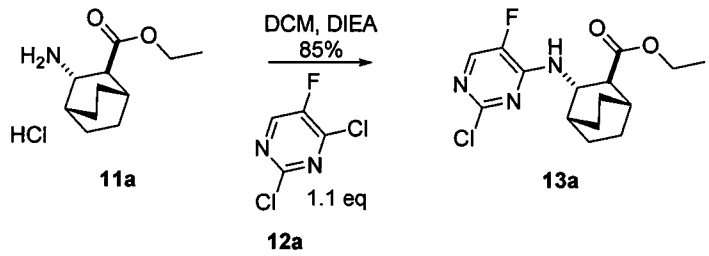
[0200] <u>*HCl in ethanol preparation:*</u> A three neck flask equipped with a temperature probe, nitrogen inlet and magnetic stirrer was charged with ethanol (1000 ml, 773 g) under a nitrogen atmosphere. The solution was stirred and cooled in a dry ice/acetone bath until an internal temperature of -12 °C was reached. Then anhydrous HCl (~ 80 g, 2.19 moles) was slowly bubbled in the cooled solution (observed temperature of -24 °C to -6 °C during addition) over 2 hours. Following the addition, the solution was transferred to a glass bottle

and allowed to warm to ambient temperature. A sample of the solution was submitted for titration giving a concentration of 2.6 M. The solution was then stored in the cold room (approximately 5 °C) overnight.

[0201] *Hydrogenation/HCl salt formation:* A glass insert to a 2 gallon Parr autoclave was charged with palladium on carbon (Pd/C (Aldrich, cat# 330108), 10 % dry basis; (50 % wet), 13.11 g, 0.01 equiv. on the basis of Compound **10a**) under a nitrogen atmosphere and then moistened with ethanol (93 g; 120 ml). Then a solution of crude Compound **10a** (212 g, 1 equiv.) in ethanol (1246 g; 1600 ml) was added to the glass insert (small rinse with ethanol to aid with transfer). The glass insert was placed in the autoclave after which HCl in ethanol (prepared as described above; 2.6 M; 1.04 equiv. based on Compound **10a**; 223 g; 259 ml) was added. The autoclave was sealed and then purged with hydrogen (3 × at 20 psi). The hydrogenation was then started under an applied pressure of hydrogen gas (15 psi) for 3 hours at which time the pressure of hydrogen appeared constant. Analysis of an aliquot of the reaction mixture by ^{1}H NMR and GC/MS indicated consumption of starting material/formation of product. The resulting mixture was then filtered over a bed of Celite (192 g) after which the Celite bed was washed with additional ethanol (3 x; a total of 1176 g of ethanol was used during the washes). The filtrate (green in color) was then concentrated under reduced pressure (water bath at 45 °C) to ~ 382 g (~435 ml); 2.9 volumes based on theoretical yield of Compound **11a.** Then isopropyl acetate (1539 g; 1813 ml (12 volumes based on theoretical yield of Compound **11a**)) was added to the remainder. The resulting solution was distilled under vacuum with gradual increase in temperature.

[0202] The distillation was stopped after which the remaining solution (370 g, ~365 ml total volume; brownish in color) was allowed to stand at ambient temperature over the weekend. The mixture was filtered (isopropyl acetate used to aid with filtration) and the collected solids were washed with additional isopropyl acetate (2 × 116 ml; each wash was approximately 100 g). The solid was then dried under vacuum at 40 °C (maximum observed temperature of 42 °C) overnight to afford 118 g (78.1 % over two steps) of Compound **11a**. The ^{1}H NMR of the material conformed to the structure of Compound **11a**, and GC/MS indicated 99% purity.

[0203] *Preparation of Compound 13a*



[0204] *Procedure A*: A mixture of 5-fluoro-2,4-dichloropyrimidine (**12a**, 39.3 g, 235 mmol, 1.1 equiv.), and HCl amine salt (**11a**, 50 g, 214 mmol) was treated with CH_2Cl_2 (169 mL) and the mixture was warmed to 30 °C. The mixture was then treated slowly with DIEA (60.8 g, 82 mL, 471 mmol, 2.2 equiv.) via syringe pump over 3 h. Peak temp was up to 32 °C. The reaction was stirred for 20 h, the reaction mixture was judged complete by HPLC and cooled to rt. The resulting reaction mixture was washed sequentially with water (211 mL, pH = 8 - 9), 5% $NaHSO_4$ (211 mL, pH = 1 - 2) then 5% aq. NaCl (211 mL, pH = 5 - 6).

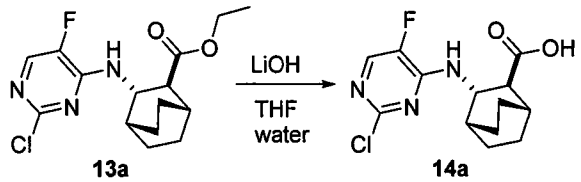
[0205] The organic phase was then distilled under reduced pressure to 190 mL. PhMe was charged (422 mL) and temperature set at 70 °C - 80 °C and internal temp at 60 °C - 65 °C until vol. back down to 190 mL. The mixture was allowed to cool to 37 °C with stirring, after approximately 10 min, crystallization began to occur and the temperature was observed to increase to 41 °C. After equilibrating at 37 °C, the suspension was charged with n-heptane (421 mL) over 3.5 h followed by cooling to 22 °C over 1 h. The mixture was allowed to stir overnight at that temperature before filtering. The resulting solid on the filter was washed with a 10% PhMe in n-heptane solution (2 × 210 mL). The solid was then dried in the oven under vacuum with an N_2 purge at 50 °C overnight. The resulting solid weighed 62 g (88% yield).

[0206] *Procedure B:* A three neck flask equipped with a mechanical stirrer, temperature probe, reflux condenser, nitrogen inlet and addition funnel was charged with Compound **11a** (51.2 g) and Compound **12a** (40.2 g) under an atmosphere of nitrogen. Dichloromethane (173 ml, 230 g) was added and the resulting mixture was stirred while warming to an internal temperature of 30 °C. Then N,N-diisopropylethylamine (85 ml, 63.09 g) was slowly added by addition funnel over 2.5-3 hours during which time an exotherm to a maximum observed temperature of 33.5 °C was observed. After complete addition, the resulting solution was stirred at 30 °C - 31 °C overnight under a nitrogen atmosphere (for approximately 19 hours).

[0207] A 100 μl sample of the reaction mixture was diluted with dichloromethane up to a total volume of 10 ml and the solution mixed well. A sample of the diluted aliquot was analyzed by GC/MS which indicated the reaction to be complete by GC/MS; observed formation of product (m/e = 328)). The reaction mixture was cooled to 26 °C and transferred to a separatory funnel (aided with dichloromethane). The mixture was then sequentially washed with water (211 ml, 211 g; pH of aqueous cut was ~8; small rag layer was transferred with aqueous cut), 5 % aqueous $NaHSO_4$ ((prepared using 50 g of sodium bisulfate monohydrate (Aldrich cat. # 233714) and 950 g water) 211 ml, 216 g; pH of aqueous cut was ~2) and then 5 % aqueous NaCl ((prepared using 50 g of sodium chloride (Aldrich cat. #

S9888) and 950 g water) 211 ml, 215 g; pH of aqueous cut was ~4-5). The collected organic phase was then concentrated under reduced pressure (water bath at 35 °C) to ~190 ml (2.7 volumes based on theoretical yield of Compound **13a** after which toluene (Aldrich cat. # 179418, 422 ml, 361 g) was added. The resulting mixture was concentrated under reduced pressure (water bath at 55 °C - 65 °C) to ~190 ml (2.7 volumes based on theoretical yield of Compound **13a**. Analysis of a sample of the solution at this stage by ^{1}H NMR indicated the absence of dichloromethane. The remaining mixture was allowed to cool to 37 °C (using water bath at 37 °C on rotovap with agitation). During this time pronounced crystallization was observed. The mixture was then mechanically stirred and heated to approximately 37 °C (external heat source set to 38 °C) after which n-heptane (430 ml, 288 g; Aldrich cat# H2198) was slowly added by addition funnel over 3 hours. Following the addition, heating was stopped and the resulting slurry mechanically stirred while cooling to ambient temperature overnight. The resulting mixture was then filtered and the collected solids were washed with 10 % toluene in n-heptane (2 × 210 ml; each wash was prepared by mixing 21 ml (16 g) of toluene and 189 ml (132 g) of n-heptane). Vacuum was applied until very little filtrate was observed. The solids were then further dried under vacuum at 50 °C under a nitrogen bleed to constant weight (3.5 hours) giving 64.7 g (90 %) of Compound **13a**. Analysis of a sample of the solid by ^{1}H NMR showed the material to conform to structure and LC analysis indicated 99.8 % purity using the supplied LC method.

[0208] *Preparation of Compound 14a*

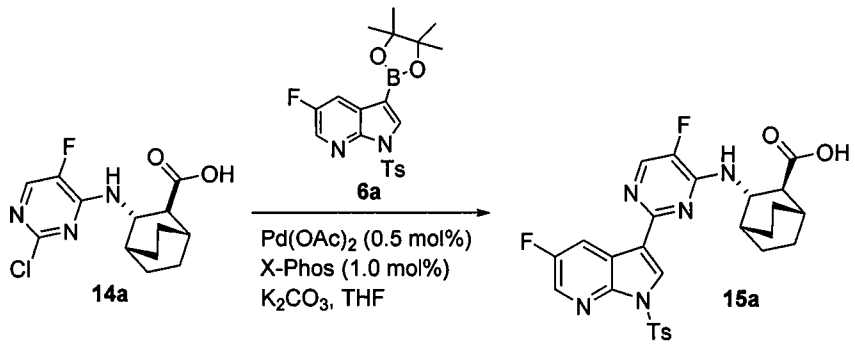


[0209] The ethyl ester **13a** (85 g, 259 mmol) was dissolved in THF (340 mL) and treated with a solution of LiOH (2M, 389 mL, 778 mmol) over 10 min (temp from 21 to 24 °C). The mixture was warmed to 45 °C with stirring for 17 h at which time the reaction was judged complete by HPLC (no SM observed). The reaction mixture was cooled to rt and CH_2Cl_2 was added (425 mL). A solution of citric acid (2 M, 400 mL) was then added slowly over 45 min (temp up to 26 °C). It was noted that during the charge some white solids were formed but quickly dissolved with stirring. The reaction mixture was stirred for an additional 15 min before phases were allowed to separate. After the phases were split, the aqueous phase pH was measured pH = 4.0. The organic phase was washed (15 min stir) with water (255 mL) –

phases were allowed to separate. The lower layer (organic) containing the desired product was then stored in the fridge overnight.

[0210] The organic phase was concentrated under reduced pressure (pot set to 65 °C) to approximately 150 mL (est. 1.76 vol. wrt SM). IPA (510 mL) was charged and distilled under reduced pressure (85 °C chiller temp setting) to 255 mL (3 vol.). The level of solvent was brought to approximately 553 mL (6.5 vol.) by the addition of IPA (298 mL). Water (16 mL) was then added and the reaction mixture warmed to reflux (77 °C) with good agitation which dissolved solids precipitated on the walls of the vessel. Reaction mixture was then cooled slowly to 65 °C (over 60 min) and held there - all material still in solution (sample pulled for residual solvent analysis). The reaction was further cooled to 60 °C and the reaction mixture appeared slightly opaque. After stirring for 15 min further cooled to 55 °C. While more product precipitates, the mixture is still thin and easily stirred. Water (808 mL) was added very slowly (2.5-3 hrs) while maintaining the temperature around 55 °C. The mixture was then cooled to 22 °C over 2 h and allowed to stir overnight. Material was then filtered and washed with a mixture of water: IPA (75:25, 2 × 255 mL). The acid was dried in a vacuum oven at 55 °C overnight. Obtained 69 g of acid **14a**, 88% yield of a white solid. The material analyzed >99% purity by HPLC.

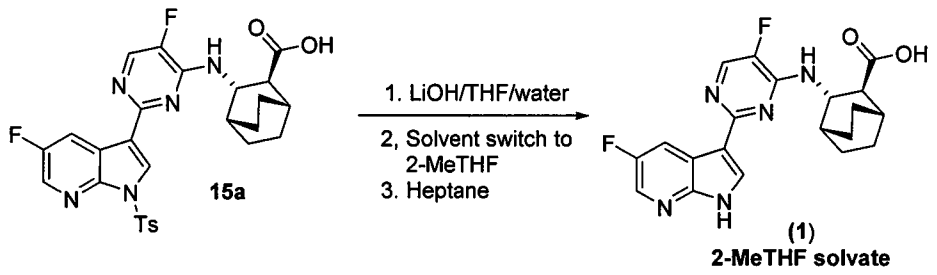
[0211] *Preparation of Compound 15a: Suzuki Coupling*



[0212] To **14a** (91.4 g, 305 mmol), **6a** (158.6 g, 381 mmol, 1.25 equiv.), $Pd(OAc)_2$ (0.34 g, 1.5 mmol, 0.5 mol%), X-Phos (1.45 g, 3.0 mmol, 1.0 mol%), and K_2CO_3 (168.6 g, 1220 mmol, 4 equiv.) was added THF (731 mL, 8 volumes) and water (29 mL, 0.32 vol). The reaction mixture was sparged with N_2 for 30 min, then warmed to 65 °C - 70 °C and stirred for 5 h. HPLC analysis of the reaction mixture showed 99.3% conversion. The reaction mixture was cooled to 22 °C - 25 °C and water was added. The mixture was stirred, the phases were allowed to separate, and the aqueous phase was decanted. A solution of 18 wt% NaCl in water (half-saturated aqueous NaCl) was added to the organic phase and the pH of the mixture was adjusted to 6.0 - 6.5 using 2N HCl. The phases were allowed to

separate and the aqueous phase was decanted. The organic phase was concentrated to a minimum volume and acetonitrile was added. The process was repeated one more time and acetonitrile was added to bring the final volume to 910 mL (10 vol). The slurry was warmed to 80 °C - 85 °C for 6 h, then cooled to 20 °C - 25 °C. The slurry was stirred for 2 h, then filtered. The solids were rinsed with acetonitrile to give **15a** (161 g, 89% yield).

[0213] *Preparation of Compound (1): Detosylation Step*



[0214] To **15a** (25 g, 45.2 mmol) was added THF (125 ml, 5 vol), then MP-TMT resin (6.25 g, 25 wt%). The mixture was stirred at 20 °C - 25 °C for 16 h and filtered, rinsing with 1 vol. THF. The resin treatment process and filtration were repeated. The THF solution was concentrated to 5 vol. To the mixture at 22 °C - 25 °C was added an aqueous solution of 2M LiOH (90.3 mL, 4 equiv.). The reaction mixture was warmed to 40 °C - 45 °C and stirred for 5 h. HPLC analysis showed 99.7% conversion. The reaction mixture was cooled to 22 °C - 25 °C and MTBE (50 mL, 2 vol) was added. Phase separation occurred. The lower aqueous phase was collected. The aqueous phase was extracted with MTBE. The lower aqueous phase was collected. To the aqueous phase was added 2-MeTHF and the mixture was stirred. The pH of the mixture was adjusted to 6.0 - 6.5, and the lower aq. phase was decanted. The organic phase was washed with pH 6.5 buffer. The organic phase was concentrated to 85 mL, diluted with 2-MeTHF (150 mL), and concentrated to a final volume of 180 mL. The resultant slurry was warmed to 70 °C - 75 °C and stirred until complete dissolution, then cooled to 45 °C - 50 °C to give slurry. The slurry was stirred for 1 h, then heptane (180 mL) was added. The slurry was cooled to 20 °C - 25 °C over 1 h and stirred for 16 h. The batch was filtered, rinsing the solids with heptane. The solids were dried to give crude Compound (**1**)•2-MeTHF solvate, 79% yield.

[0215] Example 3: Formation of Polymorphs of HCl salt of Compound (1)

[0216] *3A: Preparation of Form A of HCl salt Compound (1)•1/2H_2O*

[0217] Form A of HCl salt of Compound (**1**)•1/2H_2O was prepared by mixing 2-methyl tetrahydrofuran (2-MeTHF) solvate (1 equivalent) of Compound (**1**) (Compound (**1**)·1•(2-MeTHF)) with hydrogen chloride in a mixture of water and an organic solvent(s),

wherein the mixture of water and an organic solvent(s) had a water activity of 0.05-0.85. Particular reaction conditions employed are summarized in Table 1 below:

[0218] Table 1: Reaction Conditions Employed for the Preparation of Form A of HCl salt of Compound (**1**)•1/2H_2O.

Comp. (1) (mg) 1 (2-MeTHF)	Solvent	Solvent (mL)	Water (mL)	6N aqueous HCl (mL)	T (°C)	Eq (HCl: Compound (1))	Water (wt%)
40	Acetone	640	40	15.70	35	1.1332	8.84%
25	Acetone	400	25	9.80	46	1.1318	8.84%
10.09	Acetone	160	64	3.98	35	1.1389	32.71%
5	n-propanol	186	10	1.29	20	0.7449	6.87%
6.01	iso-propanol	88	2	2.31	35	1.1097	5.10%
6.6	iPrOH/Acetic Acid=>Acetone*	100/1.0	4	3.10	45	1.3561	7.25%
18	Acetone	180	6	3.60	30	0.5774	5.33%
18	Acetone	180	8	6.40	35	1.0266	7.73%
6	Acetone	66	11	2.82	30	1.3561	18.57%
0.101	iBuOAc	5	0.1	0.10	~20	2.8586	4.36%
6	Acetic Acid	50	8.7	2.18	35	1.0499	15.37%

*two steps: iPrOH/AcOH and then re-slurry in acetone/water

[0219] Alternatively, Form A of HCl salt of Compound (**1**)•1/2H_2O was also prepared by the following procedures: *Procedure A:* Compound (**1**)·2-MeTHF (953 g, 2.39 mol) was placed in a 30L jacketed reactor and treated with IPA (15 L) and water (0.57 L). The stirrer was started and the reaction mixture was warmed to 73 °C to get everything into solution then cooled to 50 °C - 55 °C. At 50 °C - 55 °C the reaction mixture was treated with freshly prepared HCl in IPA (0.83 M, 4.34 L) via slow addition over 4 h. It should be noted that at about the ½ way point, the mixture becomes thicker. The reaction was sampled, to check for the correct form by XRPD. After the addition, the chiller was programmed to ramp to 0 °C over 480 min with stirring. After form confirmation by XRPD analysis, the slurry was filtered into two filters. The reactor was washed with 3 L of IPA and each filter cake was washed with ~1.5 L of IPA of the IPA rinsate from the reactor. The cakes were allowed to air dry with suction overnight. The cakes were then placed in a tray dryer with no heating under vacuum with N_2 purge (22 inHg) for 24 h. Residual solvent and water analysis showed 505 ppm IPA, 8 ppm 2-Me-THF and approximately 2.15% H_2O. The material was pulled from the oven and co-milled to delump to provide 805 g of HCl salt of Compound (**1**)•1/2H_2O. *Procedure B:* Alternatively, acetone instead of IPA was used, but in a similar manner as described above in Procedure A to form HCl salt of Compound (**1**)•1/2H_2O.

[0220] The XRPD and C^{13}SSNMR data of Form A of HCl salt of Compound (**1**)•1/2H_2O are shown in FIGs 1 and 2, respectively. Certain observed XRPD peaks and C^{13}SSNMR peaks are summarized in Tables 2 and 3, respectively.

[0221] Table 2: XRPD Peaks of Form A of HCl salt of Compound (**1**)•1/2H_2O.

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
1	10.5	100.0
2	5.2	71.6
3	7.4	46.8
4	18.9	42.0
5	25.2	41.7
6	16.5	39.5
7	18.1	28.1
8	23.0	27.5
9	24.1	25.3
10	20.2	21.6
11	26.4	21.3
12	15.8	19.8
13	21.8	18.3
14	13.8	17.6
15	27.4	17.3
16	29.0	16.7
17	14.8	15.0
18	32.0	15.0
19	25.7	13.8
20	28.6	13.4
21	33.8	13.0
22	12.8	12.0
23	30.8	11.7
24	32.4	11.6
25	24.5	11.5
26	23.4	11.1
27	21.0	10.4

[0222] Table 3: C^{13} SSNMR Peaks of Form A of HCl salt of Compound (**1**)•1/2H_2O.

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
1	180.1	50.4
2	157.9	9.1
3	154.6	26.4
4	150.7	25.3
5	144.9	31.0
6	140.1	6.7
7	132.4	36.3
8	131.2	30.0

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
9	129.0	21.0
10	117.5	33.6
11	114.0	38.0
12	107.0	34.4
13	54.8	42.0
14	47.7	52.7
15	29.2	100.0
16	24.6	74.0
17	22.1	83.6

[0223] The prepared Form A of HCl salt of Compound (**1**)•1/2H_2O was found to be stable in the following solvent systems (but not limited to): chlorobenzene, cyclohexane, 1,2-dichloroethane, dichloromethane, 1,2-dimethoxyethane, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, nitromethane, tetralin, xylene, toluene, 1,1,2-trichloroethane, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, t-butylmethylether, cumene, ethanol, ethyl acetate, ethyl ether, ethyl formate, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, 2-methy-1-propanol, pentane, 1-propanol, 1-pentanol, 2-propanol, propyl acetate, tetrahydrofuran, methyl tetrahydrofuran.

[0224] Specifically, for the solubility and stability tests for Form A of HCl salt of Compound (**1**)•1/2H_2O, samples of the compound were loaded into 2 mL HPLC vials with 500 μl of solvent. The mixture was stirred at ambient temperature for 2 weeks and then filtered by centrifuge. The resulting solids were analyzed by XRPD, solutions were analyzed for solubility by quantitative NMR against hydroquinone standard. The results are summarized in Table 4.

[0225] Table 4: Summary of form and solubility data for Form A HCl salt of Compound (**1**).

Solvent	Sol. (mg/ml)	Resulting Forms
Acetonitrile	0.5	Solvate
Chlorobenzene	<0.1	A
Chloroform	<0.1	Solvate
Cyclohexane	<0.1	A
1,2-Dichloroethane	1.7	A
Dichloromethane	0.1	A
1,2-Dimethoxyethane	0.5	A
1,4-Dioxane	0.4	A
Ethylene glycol	108.1	Solvate
Hexane	<0.1	A

Solvent	Sol. (mg/ml)	Resulting Forms
Methanol	46.4	Solvate
2-Methoxyethanol	34.1	A
Methylbutyl ketone	0.4	A
Methylcyclohexane	<0.1	A
Nitromethane	<0.1	A
Tetralin	<0.1	A
Toluene	<0.1	A
1,1,2-Trichloroethane	<0.1	A
xylene	<0.1	A
Acetone	1.5	A
Anisole	<0.1	A
1-Butanol	2.9	A
2-Butanol	2.9	A
Butyl acetate	0.2	A
t-Butylmethylether	0.4	A
Cumene	<0.1	A
Dimethylsulfoxide	346.5	Solvate
Ethanol	19.9	A
Ethyl acetate	0.2	A
Ethyl ether	0.1	A
Ethyl formate	0.4	A
Formic acid	214.0	Solvate
Heptane	<0.1	A
Isobutyl acetate	0.2	A
Isopropyl acetate	0.4	A
Methyl acetate	0.6	A
3-Methyl-1-butanol	3.2	A
Methylethyl ketone	0.5	A
2-Methy-1-propanol	3.5	A
Pentane	<0.1	A
1-Pentanol	3.3	A
1-Propanol	10.7	A
2-Propanol	3.3	A
Propyl acetate	0.8	A
Tetrahydrofuran	0.7	A
Methyl tetrahydrofuran	0.7	A
Water	0.6	F

[0226] Thermogram data was obtained (the data not shown) by placing the sample in a platinum sample pan and by heating at 10 °C/min to 300 °C from room temperature. The thermogram data demonstrated a weight loss of 2.1% from 30 ° to 170 °C, which was consistent with theoretical hemihydrate (2.0%).

[0227] DSC thermogram data was obtained (the data not shown) by heating the sample at 10 °C/min to 300 °C from room temperature. DSC thermogram showed a dehydration onset

temperature of 50 °C - 100 °C followed by an onset melting/decomposition temperature of 200 °C - 260 °C.

[0228] *3B: Preparation of Form F of HCl salt Compound (1)•3H_2O*

[0229] Form F of HCl salt of Compound (**1**)•3H_2O can be prepared by slurring Form A of HCl salt of Compound (**1**)•1/2H_2O in *iso*-propanol and water, or acetone and water, or water (with a water activity value equal to, or greater than, 0.9).

[0230] For example, slurry of 100 mg of Form A of HCl salt of Compound (**1**)•1/2H_2O in 5 mL of iso-propanol/water or acetone/water at water activity of 0.9 was stirred at ambient temperature overnight. Decanting the supernatant and gentle air dry of the resulting solid material provided Form F of HCl salt of Compound (**1**)•3H_2O.

[0231] The XRPD and C^{13}SSNMR data of Form F of HCl salt of Compound (**1**)•3H_2O are shown in FIGs 3 and 4, respectively. Certain observed XRPD peaks and C^{13}SSNMR peaks are summarized in Tables 5 and 6, respectively.

[0232] Table 5: XRPD Peaks of Form F of HCl salt of Compound (**1**)•3H_2O.

XRPD Peaks	**Angle (2-Theta ± 0.2)**	**Intensity %**
1	7.1	100.0
2	9.6	83.0
3	11.9	88.8
4	12.4	84.6
5	16.4	83.5
6	17.1	83.0
7	17.5	82.8
8	19.2	86.9
9	21.1	82.2
10	21.8	83.7
11	23.9	83.8
12	28.7	83.4

[0233] Table 6: C^{13} SSNMR Peaks of Form F of HCl salt of Compound (**1**)•3H_2O.

Peak #	**Chem Shift [± 3 ppm]**	**Intensity [rel]**
1	178.6	67.6
2	156.8	21.5
3	154.3	49.3
4	152.1	12.6
5	151.2	21.3
6	142.5	37.0
7	132.3	85.7
8	127.9	15.4
9	118.0	38.6

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
10	117.5	43.7
11	115.2	36.3
12	114.5	35.2
13	106.1	15.4
14	104.8	31.6
15	52.7	43.1
16	52.3	37.2
17	48.8	44.8
18	48.4	46.4
19	30.3	100.0
20	27.4	35.4
21	25.5	37.4
22	24.5	44.5
23	23.8	40.9
24	22.0	46.4
25	21.1	47.0
26	20.7	50.5
27	20.3	47.7

[0234] A MDSC thermogram was obtained (the data not shown) by heating the sample at 2 °C/min to 350 °C from -20 °C and modulated at ± 1 °C every 60 sec. The MDSC thermogram showed a dehydration below 150 °C, melt and recrystallization between 150 °C and 200 °C, and degradation above 250 °C.

[0235] Thermogravimetric analysis (TGA) of the form was also performed. The thermogram showed a weight loss of 12% up to 125 °C which was close to theoretical trihydrate (11%). The second step weigh loss below 200 °C was indicated by TGA-MS to be the loss of HCl. The melting/ decomposition onset was around 270-290 °C.

[0236] *3C: Preparation of Form D of HCl salt Compound (1)*

[0237] Anhydrous Form D of HCl salt of Compound (**1**) can generally be made by dehydrating Form A of HCl salt of Compound (**1**)•1/2H_2O. The dehydration could be done via heating or dry nitrogen purge, or the combination of the two. For example, 2 mg of Form A of HCl salt of Compound (**1**)•1/2H_2O was heated on a hot plate, generating the desired anhydrous Form D at approximately 85 °C.

[0238] The XRPD and C^{13} SSNMR data of anhydrous Form D of HCl salt of Compound (**1**) are shown in FIGs 5 and 6, respectively. Certain observed XRPD peaks and C^{13} SSNMR peaks are summarized in Tables 7 and 8, respectively.

[0239] Table 7: XRPD Peaks of Form D of Anhydrous HCl salt of Compound (1).

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
1	5.3	100.0
2	10.5	56.0
3	15.9	49.2
4	25.9	30.5
5	21.0	24.6
6	26.5	24.1
7	5.8	22.6
8	7.4	21.7
9	19.0	17.4
10	16.6	17.2
11	25.3	16.1
12	24.7	16.0
13	29.4	15.5
14	13.8	14.6
15	20.3	14.5
16	32.0	14.4
17	19.5	12.4
18	28.6	12.4
19	17.1	11.5
20	30.3	11.4
21	27.5	11.0
22	27.0	10.7
23	23.7	10.4
24	28.0	10.2
25	21.6	10.1

[0240] Table 8: C^{13} SSNMR Peaks of Form D of Anhydrous HCl salt Compound (1)

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
1	179.7	43
2	177.8	44.85
3	157.5	16.88
4	154.9	43.14
5	151.1	25.79
6	149.8	21.51
7	145.0	26.82
8	143.9	35.41
9	141.6	14.85
10	139.7	12.9
11	135.4	29.94
12	132.5	43.37
13	130.1	23.65
14	128.9	27.35
15	127.3	25.35

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
16	118.1	27.24
17	116.6	28.25
18	113.3	52.71
19	107.5	29.33
20	106.1	30.73
21	54.4	39.43
22	53.4	42.25
23	48.2	54.53
24	47.2	47.8
25	31.6	52.54
26	29.4	100
27	26.0	50.37
28	24.8	47.38
29	23.9	63.88
30	22.9	98.06
31	20.2	45.7

[0241] *3D: Water Activity Tests*

[0242] A competition slurry study of Form A of HCl salt of Compound (**1**)•1/2H_2O seeded with Form F of HCl salt of Compound (**1**)•3H_2O, at water activities of 0.0 to 0.8 of isopropyl alcohol/water showed that Form A to be the most stable form among Form D of anhydrous HCl salt Compound (**1**) Form F of HCl salt of Compound (**1**)•3H_2O, and Form A of HCl salt of Compound (**1**)•1/2H_2O, after approximately 2 weeks of stirring under ambient conditions. At an IPA/water activity of 0.9, Form A of HCl salt of Compound (**1**)•1/2H_2O was converted to Form F of HCl salt of Compound (**1**)•3H_2O. The results from these studies are summarized in Table 9 below.

[0243] Table 9: Water Activity Tests on HCl salt of Compound (**1**)•1/2H_2O in IPA/water mixtures.

Starting Forms	Water Activity (a_w)	Water wt%	Final Form	Description
A+F	0 + >80 °C		D	Anhydrate
A+F	0		A	Hemihydrate
A+F	0.1	0.1	A	Hemihydrate
A+F	0.2	0.25	A	Hemihydrate
A+F	0.3	0.35	A	Hemihydrate
A+F	0.4	0.55	A	Hemihydrate
A+F	0.5	0.75	A	Hemihydrate

Starting Forms	Water Activity (a_w)	Water wt%	Final Form	Description
A+F	0.6	1.00	A	Hemihydrate
A+F	0.7	1.35	A	Hemihydrate
A+F	0.8	1.85	A	Hemihydrate
A+F	0.9	2.80	F	Trihydrate
A+F	1	100	F	Trihydrate

[0244] A phase diagram of temperature against water activity for the transition among Form D of anhydrous HCl salt Compound (**1**) ("Form D"), Form F of HCl salt of Compound (**1**)•$3H_2O$ ("Form F), and Form A of HCl salt of Compound (**1**)•$1/2H_2O$ ("Form A") is shown in FIG. 12.

[0245] *3F: Amorphous HCl salt of Compound (1)*

[0246] Amorphous HCl salt of Compound (**1**) could be formed by treating Me_2NEt salt of Compound (**1**) (1.985 g) in water and 2-MeTHF with 1.05 eq. NaOH, followed by treatment with HCl to remove amine and crash out from an aqueous layer (pH 2-3). The resulting slurry was concentrated to remove any organics and then filtered. The resulting solid was rinsed with small portions of water and dried. Me_2NEt salt of Compound (**1**) was prepared according to WO 2010/148197, followed by usual chiral separation and purification: SCF chiral chromatography with a modifier that included Me_2NEt (which generated Me_2NEt salt of Compound (**1**)).

[0247] Example 4: Formation of Polymorphs of Free base Compound (1)

[0248] *4A: Preparation of Form A of Free Base Compound (1)*

[0249] Form A of free base Compound (**1**) was produced by the following procedure: Crude amorphous free base Compound (**1**) (approximately 135g) was transferred to a 4L jacketed reactor and the reactor was charged with ethanol (2.67 L) and water (0.325 L) (10% water solution). The mixture was heated to reflux. Water (300 mL) was added to the resulting mixture of step 2) to make a 20% water solution. The resulting mixture was then cooled to 55°C (rate= -1°C/min) and subsequently held for 30 minutes. Crystalline seed of free base Form A of Compound (**1**) (1.5 g, 3.756 mmol) was then added into the cooled mixture, and the resulting mixture was held for 30 minutes while the product precipitated. The seed of crystalline free base Form A of Compound (**1**) was produced by slurrying amorphous free base Compound (**1**) (20 mg) in nitromethane (0.5 mL). Additional seed materials of crystalline free base Form A of Compound (**1**) were produced by slurring amorphous free

base Compound (**1**) (900 mg) in acetonitrile (10 mL) with the seed obtained using nitromethane. Into the mixture containing the seed of crystalline free base Form A of Compound (**1**) was slowly added water (795.0 mL) to make a 40% water solution. The resulting mixture was cooled down slowly to 0 °C (~ -10°C/hour), and subsequently held for 2 hours. Solid materials were then filtered and air dried, and then further dried in oven at 60°C for 18 hours.

[0250] Alternatively, 2-methyl THF solvate of free base Compound (**1**) instead of amorphous free base Compound (**1**) was used and Form A of free base Compound (**1**) was also obtained in a similar matter as described above.

[0251] The prepared Form A of Compound (**1**) was found to be stable in the following solvent systems (but not limited to): acetonitrile, chlorobenzene, chloroform, cyclohexane, 1,2-dichloroethane, dichloromethane, 1,2-dimethoxyethane, ethylene glycol, formamide, hexane, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidinone, nitromethane, tetralin, toluene, 1,1,2-trichloroethane, acetic acid, anisole, 1-butanol, butyl acetate, cumene, ethyl acetate, ethyl ether, ethyl formate, heptane, isobutyl acetate, isopropyl acetate, 3-methyl-1-butanol, 2-methy-1-propanol, pentane, propyl acetate, water, water-iso-propanol (1:3 vol/vol), and water-acetonitrile (1:1 vol/vol; 1:3 vol/vol).

[0252] The XRPD and C^{13} SSNMR Data of Form A of Compound (**1**) are shown in FIGs 7 and 8, respectively. Certain observed XRPD peaks and C^{13} SSNMR peaks are summarized in Tables 10 and 11, respectively.

[0253] Table 10: XRPD Peaks of Form A of Compound (**1**)

XRPD Peaks	**Angle (2-Theta ± 0.2)**	**Intensity %**
1	11.8	100.0
2	18.9	100.0
3	16.9	99.8
4	15.5	99.7
5	22.0	99.7
6	25.5	99.7
7	9.1	99.4
8	23.6	98.6
9	27.6	98.5
10	17.5	98.3
11	23.0	98.3
12	24.0	98.3
13	13.7	98.2
14	20.2	98.2
15	12.5	97.8
16	10.6	97.7

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
17	15.8	97.5
18	20.6	97.5
19	12.9	97.4
20	24.7	97.4
21	26.2	97.4
22	6.2	97.3
23	21.1	97.3

[0254] Table 11: C^{13} SSNMR Peaks of Form A of Compound (**1**)

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
1	180.0	60.1
2	176.2	68.7
3	175.9	62.4
4	160.2	28.8
5	158.6	18.4
6	157.9	28.1
7	157.3	47.2
8	156.0	34.3
9	155.4	49.7
10	152.3	32.5
11	151.4	49.5
12	146.5	18.6
13	144.4	61.1
14	143.8	56.4
15	142.9	19.2
16	140.2	21.2
17	138.5	55.6
18	133.6	29.4
19	132.3	61.4
20	131.0	52.1
21	126.2	23.0
22	121.5	35.8
23	120.8	39.3
24	119.7	90.9
25	116.2	59.3
26	115.3	44.3
27	112.7	35.0
28	52.5	39.0
29	51.6	75.9
30	50.4	94.8
31	49.8	74.6
32	31.8	80.4
33	31.2	53.0
34	30.5	86.0
35	30.1	95.1

Peak #	Chem Shift [± 3 ppm]	Intensity [rel]
36	28.5	100.0
37	26.3	81.0
38	25.9	96.1
39	25.0	82.2
40	22.8	66.97
41	22.2	55.41
42	21.6	64.44
43	21.0	82.87
44	20.4	57.45
45	19.8	52.2

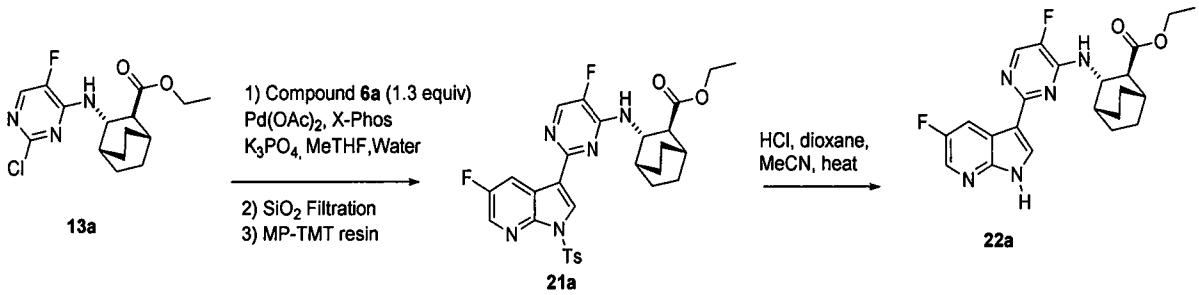
[0255] Thermogravimetric analysis of the product, Form A of Compound (**1**), was performed (the data not shown here) on the TA Instruments TGA model Q500 by placing a sample of it in a platinum sample pan and by subsequent heating the pan at 10 °C/min to 300 °C from room temperature. The thermogram demonstrated a decomposition onset was around 293 °C.

[0256] A DSC thermogram for Form A of Compound (**1**) was also obtained using TA Instruments DSC Q200. A sample of the form was heated at 10 °C/min to 350 °C. The DSC thermogram showed the melting temperature to be around 278 °C.

[0257] *4B: Preparation of Form B of Hydrates of Free Base Compound (1)*

[0258] A hydrated form of free base Compound (**1**) was isomorphic as Form A of free base Compound (**1**). Form A of free base Compound (**1**) could freely convert to the hydrated form B when it was exposed to high humidity and revert back when the humidity was lowered. According to the phase changes determined using DSC experiments (data not shown), the transition temperature was close to ambient temperature and varied with water activity. For example, at ambient temperature, the hydrate form was observed where a water activity was greater than 0.6, such as 0.6-1.0.

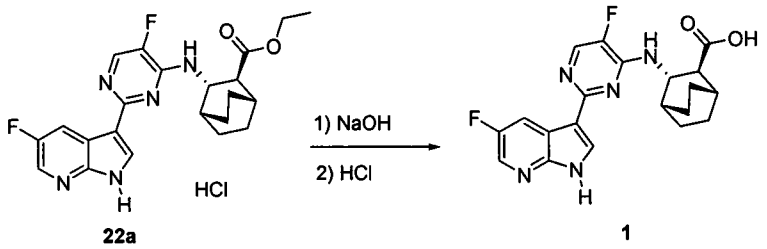
[0259] *4C: Preparation of Amorphous Free Base Compound (1)*



[0260] Suzuki coupling was performed by taking up the chloropyrimidine, Compound **13a**, boronic ester Compound **6a**, catalyst $Pd(OAc)_2$, and ligand (X-phos) in 10 vol. of 2-MeTHF.

This mixture was heated to 65 °C and 2 vol. of a 50% aqueous solution of K_3PO_4 were added at a rate that maintained the reaction mixture at 65 °C. Both reactions went to full conversion then were cooled to 20 °C and filtered through celite. The aqueous layers were separated to waste, the organic layers washed with 5% aqueous NaCl, and then concentrated to dryness to give approximately 3.5 kg of a dark green paste for each. The crude oil was divided into 4 equal portions, slurried with 400 g of SiO_2 and 500 g of Florisil, and eluted through a 2.3 kg SiO_2 column with heptane/EtOAc (5:1 to 3:1, 2L fractions) combining all product containing fractions. These fractions were concentrated to dryness to give approximately 2.9 kg of Compound **21a**.

[0261] Compound **21a** was dissolved in 10 vol. (25 L) of CH_3CN and treated with 4 eq. of HCl (4.31 L of 4N HCl in 1, 4-dioxane) at 70 °C for 15 h. The reaction was judged 100% complete by HPLC and the thin slurry cooled to 20 °C in 1h. TBME (28 L, 11 vol) was added at 0.5 L/min with the slurry becoming very thick (gelatinous) at the end of the addition. After 4-5 h stirring, the slurry became much thinner. The resulting solids were collected by suction filtration and washed with 3 × 5 L TBME giving a low density cake, and dried under a N_2 steam for 3 days to give 1.71 kg (86 % yield, 98.9% AUC purity) of Compound **22a**•HCl.



[0262] A solution of NaOH (55.60 mL of 2M, 111.2 mmol) was added to a suspension of Compound **22a**•HCl (10g, 22.23 mmol) in 2-MeTHF (100.00 mL) at 20 °C. The reaction mixture was stirred at 60 °C for 5 h, and then additionally at 67 °C. After about 22 hours' stirring, 100 mL (10 vol) of 2-MeTHF was added to the resulting mixture. The batch was then cooled to 0 °C. HCl was added to the resulting mixture to adjust the pH to pH 6.6 to produce crude free base Compound (**1**). The crude material in 60 mL (6 vol) of 2-Me-THF was heated to 50 °C. 50 mL (5 vol) of n-heptane was added into the resulting mixture over 1 hour. The batch was then cooled to 20 °C. The solid product was filtered, and the solid product was further purified by column chromatography (EtOAc/heptane 2:1 to 4:1). Its XRPD data indicated amorphous free base Compound (**1**).

[0263] Alternatively, amorphous free base Compound (**1**) was observed from a mixture of Form A of free base Compound (**1**) and a solvent selected from 2-ethoxyethanol,

2-methoxyethanol, t-butylmethylether, formic acid, or methylethyl ketone (e.g., see Table 13 below), which was stirred at ambient temperature.

[0264] *4D: Preparation of 2-MeTHF Solvate of Free Base Compound (1)*

[0265] Compound (**1**) •1(2-MeTHF) was prepared as described in Example 2 above. Its XRPD data is shown in FIG. 10. Certain observed XRPD peaks are summarized in Table 12.

[0266] Table 12: XRPD Peaks of Compound (**1**)•1(2-MeTHF).

XRPD Peaks	**Angle (2-Theta ± 0.2)**	**Intensity %**
1	6.4	9.78
2	8.4	38.07
3	9.7	43.96
4	12.9	15.57
5	16.7	100
6	16.9	46.55
7	17.4	18.67
8	19.4	16.54
9	20.0	14.62
10	21.0	20.4
11	21.3	13.58
12	22.3	37.59
13	24.3	15.36
14	25.7	16.34
15	25.9	10.06

[0267] *4F: Solubility and Stability Data of Form A of Free Base Compound (1) and Amorphous Compound (1) in Various Solvent Systems*

[0268] Solubility and stability of Form A free base Compound (**1**) ("Form A") and amorphous compound (**1**) ("amorphous") in in various solvent systems were tested at ambient temperature in a similar manner as described above for those of Form A of HCl salt of Compound (**1**). The resulting data are summarized in Table 13.

[0269] Table 13: Solubility and Stability Data of Form A free base Compound (**1**) ("Form A") and amorphous compound (**1**) ("Amorphous")

	Starting Form A		**Starting Amorphous**
Solvent	**Sol. (mg/ml)**	**Resulting Form**	**Resulting Form**
Acetonitrile	1.0	A	Amorphous
Chlorobenzene	0.4	A	Amorphous
Chloroform	3.8	A	Amorphous
Cyclohexane	<0.1	A	Amorphous

	Starting Form A		Starting Amorphous
Solvent	**Sol. (mg/ml)**	**Resulting Form**	**Resulting Form**
1,2-Dichloroethane	0.4	A	Amorphous
Dichloromethane	0.9	A	Amorphous
1,2-Dimethoxyethane	114.0	A	Amorphous
N,N-Dimethylacetamide	>150	Solvate	Solvate
N,N-Dimethylformamide	39.2	Solvate	No signal
1,4-Dioxane	21.3	Solvate (1:1)	Solvate (1:1)
2-Ethoxyethanol	>113	Amorphous	No signal
Ethylene glycol	10.4	A	Solvate
Formamide	7.0	A	Amorphous
Hexane	<0.1	A	Amorphous
Methanol	25.5	Solvate	Solvate
2-Methoxyethanol	>114	Amorphous	No signal
Methylbutyl ketone	20.0	A	Amorphous
Methylcyclohexane	<0.1	A	Amorphous
N-Methylpyrrolidinone	>149	A	No signal
Nitromethane	0.3	A	Amorphous
Tetralin	<0.1	A	Amorphous
Toluene	0.3	A	Amorphous
1,1,2-Trichloroethane	1.0	A	Amorphous
xylene	0.3	Solvate	Amorphous
acetic acid	42.8	A	Solvate
Acetone	16.3	Solvate	Solvate
Anisole	0.7	A	Amorphous
1-Butanol	21.0	A	Solvate (1:1)
2-Butanol	14.0	Solvate (1:1)	Solvate(1:1)
Butyl acetate	8.1	A	Amorphous
t-Butylmethylether	10.4	Amorphous	Amorphous
Cumene	0.3	A	Amorphous
Dimethylsulfoxide	>113	No signal	No signal
Ethanol	35.5	No signal	A
Ethyl acetate	11.6	A	Amorphous
Ethyl ether	3.5	A	Amorphous
Ethyl formate	8.1	A	Solvate(1:1)
Formic acid	>89.4	Amorphous	No signal
Heptane	<1.5	A	Solvate
Isobutyl acetate	4.4	A	Amorphous
Isopropyl acetate	6.2	A	Amorphous
Methyl acetate	9.4	Solvate	Solvate
3-Methyl-1-butanol	9.7	A	Solvate
Methylethyl ketone	27.3	Amorphous	Solvate(1:1)
2-Methy-1-propanol	12.2	A	Solvate(1:1)
Pentane	<0.3	A	Amorphous
1-Pentanol	14.5	No signal	Solvate(1:1)

	Starting Form A		Starting Amorphous
Solvent	**Sol. (mg/ml)**	**Resulting Form**	**Resulting Form**
1-Propanol	15.9	Solvate	No signal
2-Propanol	12.9	Solvate(1:1)	Solvate(1:1)
Propyl acetate	7.5	A	Amorphous
Tetrahydrofuran	61.2	Solvate(1:1)	Solvate(1:1)
Methyl tetrahydrofuran	34.8	Solvate(1:1)	Solvate(1:1)
Water	<0.1	A	Amorphous
Water-IPA 1:1	-	Solvate	-
Water-IPA 1:3	-	A	-
Water-ACN 1:1	-	A	-
Water-ACN 1:3	-	A	-
Water-MeOH 1:1	-	Solvate	-
Water-MeOH 1:3	-	Solvate	-

[0270] Example 5: Preparation of Form A of Tosylate Salt of Compound (1)

[0271] Form A of tosylate salt of Compound (**1**) was prepared by slurring amorphous free base Compound (**1**) (500 mg) and *p*-toluenesulfonic acid in acetonitrile (20 ml). Samples were stirred overnight. Its XRPD data are shown in FIG. 9. Certain observed XRPD peaks are summarized in Table 14.

[0272] Alternatively, 2-methyl THF solvate of free base Compound (**1**) instead of amorphous free base Compound (**1**) could be used to prepare Form A of tosylate Compound (**1**) in a similar matter as described above.

[0273] Table 14: XRPD Peaks of Form A of Tosylate Salt Compound (**1**)

XRPD Peaks	**Angle (2-Theta ± 0.2)**	**Intensity %**
1	6.0	30.21
2	7.2	100
3	9.3	37.8
4	12.9	13.96
5	13.7	39.23
6	14.3	50.25
7	14.7	42.94
8	16.4	9.99
9	16.9	89.79
10	18.7	59.65
11	19.3	19.62
12	19.6	33.34
13	20.3	11.38
14	20.8	11.98

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
15	21.9	41.6
16	23.0	33.45
17	24.2	14.97
18	25.4	23.83
19	26.3	44.54
20	26.9	51.79
21	27.5	34.02
22	28.0	36.07
23	29.1	13.36
24	29.7	8.92
25	32.2	9.25
26	33.1	4.75

[0274] Example 6: Formulations of Compound (1)

[0275] A. Tablets of Compound **(1)**

[0276] *Compositions*

[0277] Form A of HCl salt of Compound (**1**)•1/2H_2O (hereinafter simply Compound (**1**) for Example 6) was employed for the tablet formation. All excipients complied with the current monographs of the European Pharmacopoeia and the USP/NF and are purchased from approved suppliers.

[0278] The formulation composition and batch size for the pre granulation blend and the granulation binder solution are given in Table 15A. The batch size of the binder solution included a 100% overage for pump calibration and priming of solution lines. The theoretical compression blend composition is also given in Table 15A. The actual quantities for the batch were calculated based on the yield of the dried granules. The composition and approximate batch size of the film coating suspension is given in Table 15B and included 100% overage for pump calibration and priming of suspension lines. The target amount of the film coating was 3.0% w/w of the tablet weight.

[0279] Table 15A: Compositions of Tablets of Compound (**1**).

		% in pre-granulation blend	% in dry granule	% in tablet core	mg in tablet (300 mg)
Intra granular	Compound (1) crystalline hemihydrate, HCl salt (Form A)	76.13	74.99	50.00	333.00
	Avicel PH-101, NF, PhEur	10.03	9.88	6.59	43.89

		% in pre-granulation blend	% in dry granule	% in tablet core	mg in tablet (300 mg)
	Lactose Monohydrate, #316, NF, PhEur	10.03	9.88	6.59	43.89
	Ac-Di-Sol, NF, PhEur, JP	3.81	3.75	2.50	16.65
	total pre-granulation blend:	100.00	98.50	65.68	437.43
In binder solution	Povidone K30, USP		1.50	1.0	6.66
	Water, USP		na	na	na
	total granules:		100.00	66.68	444.09
Extra granular	Prosolv 50, NF			28.82	191.94
	Ac-Di-Sol, NF, PhEur, JP			2.50	16.65
	SSF, NF			2.00	13.32
	Total core tablet			100	666.00
In film coating suspension	Opadry II, 85F18422			(3.2 wrt core)	21.31
	Water, USP				na
	Total final coated tablet				**687.31**

[0280] Table 15B: Film coat suspension composition and approximate batch size.

Component	% W/W	Batch size (g)
Opadry II White, 33G	15.00	210.00
Water, USP	85.00	1190.00
Total	**100.00**	**1400.00**

[0281] *Binder Solution preparation*

[0282] The binder solution consisted of Povidone and water. The solution was prepared based on 40% water content in the final granulation. Thus, the total amount of solids in solution (Povidone) was 3.6% (w/w). An excess amount of 100% was prepared for priming lines, etc. Based on visual inspection of startup of the granulation run, additional stock solutions of +/- 2% (38-42%) water in the final granulation was prepared. Typically, 87.00 g Povidone K30, and 2320.00 g purified (DI) water were weighed, and under constant stirring was added the Povidone K30 into the container containing the DI water. After the addition, the container was sealed to minimize evaporation, and the solution was stirred until all the solids present were fully dissolved.

[0283] *Wet granulation process flow*

[0284] Wet granulation was performed by the procedures described below: Excess (10%) amount of Compound (**1**), Avicel PH-101, Fastflo lactose and Cross Carmellose Sodium were weighed (see Table 15A). They were screened using a 20 mesh hand screen or a cone mill

equipped with an 813 μm grated mesh screen at 1000 rpm (for a U5 Quadro Co-mill). The screened materials were placed in individual bags or containers. The materials were then transferred into a blender, and were blended for 15 minutes at typically 15 RPM. The blended materials were milled using U5 Quadro cone mill equipped with 4mm square hole screen at 1000 rpm. The milled materials were blended again, repeating the blend step. The re-blended materials were then fed into a twin screw granulator. The bulk wet granulation was fed into the granulator using a Loss in Weight feeder (K-tron or similar). The resulting materials were then granulated. The binder fluid (see Table 15A) was injected into the twin screw granulator using a peristaltic pump. The ratio of solution feed rate over powder feed rate was 0.4095. For example, if the powder feed rate was 15.00 g/min, the solution feed rate was 0.4095*15.00 = 6.14 g/min, with a water content of 40% (based on the dry mass). The granule sub batches were collected into pre-tared drying trays. The collected materials were evenly sprayed on a tray and dry the material in an oven to form dried granules. The dried granules were placed into K-tron to starve feed continuously into cone mill and subsequently milled.

[0285] *Extra-granular blending and compression process*

[0286] Extra-granular blending and compression process were performed by the procedures described below: The quantity of the extra-granular excipients based on the compression blend composition was weighed. The weighed excipients were screened using a U5 Comil with a 32C screen and round bar impeller at 1000 rpm. The milled granules of Compound (**1**) were first added to the blender containing the screened Avicel PH-102 and Ac-Di-Sol. They were blended for 8 minutes at 16 RPM. Sodium stearyl (SSF) was screened through a mesh 50 hand screen into an appropriate container. A portion of the extra granular blend equal to roughly 10 times by mass the amount of SSF was placed in the container with the SSF and bag blend for 30 seconds before adding the mixture to the bin blender. All of the materials were then blended for 2 minutes at 16 rpm. The final blend was then compressed according to the prescribed tablet compression process parameters.

[0287] *Film coating process*

[0288] A film coating was applied to the core tablets in a Vector VPC 1355 pan coater as a 15 % w/w Opadry II white # 33G aqueous suspension. The target coating was 3.0% w/w of the core tablet weight, with an acceptable range of 2.5% to 3.5%. To accomplish this, an amount of coating suspension equivalent to a 3.2% weight gain was sprayed, which gave a 3.0% coating assuming a coating efficiency of 95%.

[0289] B. Intravenous (IV) Formulations of Compound (1)

[0290] Form A of HCl salt of Compound (1)•1/2H_2O (hereinafter simply Compound (1) for Example 6) was supplied as a 2 mg/mL solution for intravenous (IV) administration. The composition of the solution along with the quality reference and function of each component were provided in Tables 16A and 16B.

[0291] Table 16A: Composition of the Solution Vehicle[a].

Component	Quality Standard	Component Function	Amount (mg/50g IV solution)	Content (% w/w)
Sodium Phosphate monobasic, anhydrous	USP	Buffering agent	26	0.052
Sodium Phosphate dibasic, heptahydrate	USP	Buffering agent	1281	2.562
Dextrose, anhydrous	USP	Tonicity modifier	500	1.000
Water for injection	USP	Solvent	48,193	96.386
Total	--	--	**50,000**	**100%**
Abbreviations: USP, United States Pharmacopoeia				

[a] Solution will be adjusted for pH with NaOH or HCl

[0292] Table 16B: Composition of Compound (1) Intravenous Solution[a].

Component	Component Function	Amount (mg/50g IV solution)	Content (% w/w)
Compound (1)[b]	Drug substance	111	0.222
Solution Vehicle (from Table 1)	Solvent	49,889	99.778
Total	--	**50,000**	**100%**

[a] Solution was adjusted for pH with NaOH or HCl. Density of solution is 1.000 g/cm^3.

[b] The drug substance was a hemihydrate HCl salt. The amount of drug substance was calculated based on the active anhydrous free base equivalent, where a conversion factor from the free base to the hemihydrate HCl salt is 1.11.

[0293] Example 7: In Vivo Assay for Combination of Compound (1) With or Without Oseltamivir

[0294] Infected mice were treated with vehicle or escalating dose levels of Form A of HCl salt of Compound (1)•1/2H_2O in combination with the clinically relevant dose of Oseltamivir starting 48 hours post influenza A challenge or 2 hours prior to Influenza B challenge.

[0295] *Methods*: In these studies, Form A of HCl salt of Compound (**1**) hemihydrate (hereinafter simply Compound (**1**) for Example 7) was formulated in a vehicle containing 0.5% (w/v) MC (Sigma-Aldrich, St Louis, MO), yielding a homogeneous suspension, and the dose of the compound was based upon the HCl salt of Compound (**1**) hemihydrate. Oseltamivir was formulated in distilled deionized water yielding a homogeneous suspension. The combination of Compound (**1**) with oseltamivir was formulated in a vehicle containing 0.5% (w/v) MC. The combination formulations were prepared at the beginning of each study and stored at 4 °C for up to 10 days with stirring in the dark. All formulations and vehicles were administered to mice via oral gavage at a dosing volume of 10 mL/kg.

[0296] Male Balb/c mice (5-7 weeks, 17-19 grams) were anesthetized and inoculated with a lethal dose of mouse-adapted influenza virus A/PR/8/34 or B/Mass/3/66 by intranasal instillation. Eight mice were enrolled per study group. Treatments were initiated +48 hours post inoculation for influenza A or 2 hours prior to inoculation for influenza B. Vehicle (10 mL/kg) and Compound (**1**) at doses of 0.1 - 10 mg/kg was administered alone or in combination with 10 mg/kg Oseltamivir orally (PO) twice daily (BID) for 10 days in the influenza A study. Vehicle (10 mL/kg) and Compound (**1**) at doses of 1 - 10 mg/kg was administered alone or in combination with 10 mg/kg Oseltamivir orally (PO) twice daily (BID) for 10 days in the influenza B study. Mice were weighed and observed daily for signs of morbidity for 21 days after infection. In addition lung function was monitored by unrestrained WBP (Buxco, Troy, NY).

[0297] Influenza A/PR/8/34 (VR-1469) and Influenza B/Mass/3/66 (VR-523) were obtained from ATCC (Manassas, VA). Stocks were prepared by standard methods known in the art. Briefly, virus was passaged at low multiplicity of infection in Madin-Darby canine kidney cells (MDCK cells, CCL-34, ATCC), the supernatant harvested after approximately 48 hours and centrifuged at 650 × g for 10 minutes. Virus stocks were frozen at -80 °C until used. Virus titers ($TCID_{50}$/ml) were calculated by the Spearman-Karger method after serially diluting the virus sample, infecting replicate MDCK cultures, and measuring the cytopathic effect (CPE) based on ATP content at 96 hours (CellTiter-Glo, Promega, Madison WI).

[0298] Mice were weighed daily for 21 days after infection. Body weight data were analyzed using Two Way ANOVA and a Bonferroni post test to compare groups. P-values less than 0.05 were considered significant.

[0299] Mice were observed daily for 21 days post influenza infection. Any mouse that scored positive for four of the following six observations (>35% BW loss, ruffled fur, hunched posture, respiratory distress, reduced mobility, or hypothermia) was deemed

moribund, then euthanized and scored as a death in accordance with guidelines established with the Vertex Institutional Animal Care and Use Committee. Survival data were analyzed using the Kaplan Meier method.

[0300] Mice were subjected to unrestrained WBP (Buxco, Troy, NY). Lung function is expressed as enhanced pause (Penh), a unit-less calculated value that reflects pulmonary resistance. This value is derived from changes in the holding container pressure that fluctuates as a consequence of changes in the animal's breathing pattern. Bronchoconstriction of the animal's airways will affect the flow of air and, hence, pressure in the holding container. The changes in pressure are tracked during expiration (PEP) and inspiration (PIP). Penh values were calculated according to the formula Penh = pause x PEP/PIP, where "pause" reflects the timing of expiration. Mice were acclimated in the Plethysmography chamber for 15 minutes, then data were collected in one minute intervals, averaged over 10 minutes, and expressed as absolute Penh values. Data were analyzed using Two Way ANOVA and a Bonferroni post test to compare groups. P-values less than 0.05 were considered significant.

[0301] *Results:* Compound (**1**) was evaluated in combination with Oseltamivir for its ability to prevent mortality and morbidity, reduce BW loss, and prevent and/or restore lung function in a murine model of influenza pulmonary infection versus Compound (**1**) or Oseltamivir treatment alone. The combination showed no deleterious effect on the efficacy of each of the drugs as compared to each drug administered alone. In addition, the combination treatment showed synergy in influenza A treatment as the failure dose for each compound alone (0.3 and 10 mg/kg of Compound (**1**) and Oselatamivir, respectively) when combined increased survival from 0 to 100 percent. Compound (**1**) has little activity against influenza B in vivo (as expected from available in vitro data) and does not interfere with the effectiveness of Oseltamivir.

[0302] *Influenza A mouse model*: All of the vehicle-treated controls succumbed to disease by days 9 or 10. Treatment at 1, 3 and 10 mg/kg Compound (**1**) BID alone provided complete protection from death, reduced BW loss and restored lung function when dosing was initiated +48 hours post infection as compared to vehicle controls (Table 17). Treatment at 0.1 and 0.3 mg/kg Compound (**1**) and 10 mg/kg Oseltamivir administered alone did not protect from death reduce BW loss or restore lung function when treatment initiated +48 hours post influenza A infection. Interestingly, 0.3mg/kg Compound (**1**) and Oseltamivir administered together +48 hours post influenza A infection provided complete protection from death, reduced BW loss and restored lung function.

[0303] Table 17: In Vivo Efficacy Data of Compound (**1**) with or without Oseltamivir Administered + 48 Hours After Influenza A Infection.

Compound (1)/Oseltamivir Combination in FluA						
Oseltamivir mg/kg	**0**			**10**		
Compound (1) mg/kg	**Survival (21 days) (%)**	**Weight Loss (Day 8) (%)**	**Penh (Day 3)**	**Survival (21 days) (%)**	**Weight Loss (Day 8) (%)**	**Penh (Day 3)**
0	0	33.9	2.28	0	32.0	2.36
0.1	0	34.2	2.15	0	31.6	2.09
0.3	0	32.4	1.90	100	29.3	1.80
1	100	28.2	2.11	100	23.4	1.23
3	100	22.2	1.68	100	17.6	1.11
10	100	14.6	0.95	100	8.4	0.79

[0304] *Influenza B mouse model*: All of the vehicle-treated controls succumbed to disease by days 7 or 8. Administration of 1, 3, or 10 mg/kg Compound (**1**) alone -2h prior to influenza B infection and continued BID for 10 days provided no significant protection against morbidity, BW loss or loss of lung function as compared to controls. Oseltamivir administered at 10 mg/kg alone or in conjunction with 1, 3 or 10 mg/kg Compound (**1**) -2h prior to influenza B infection provided complete protection from death, reduced BW loss and restored lung function (Table 18).

[0305] Table 18: In Vivo Efficacy Data of Compound (**1**) with or without Oseltamivir Administered + 48 Hours after Influenza B Infection

Compound (1)/Oseltamivir Combination in FluB						
Oseltamivir mg/kg	**0**			**10**		
Compound (1) mg/kg	**Survival (21 days) (%)**	**Weight Loss (Day 8) (%)**	**Penh (Day 6/7)**	**Survival (21 days) (%)**	**Weight Loss (Day 8) (%)**	**Penh (Day 6/7)**
0	0	ND	2.20	100	12.8	1.08
1	0	33.6	1.90	100	7.7	1.26
3	0	33.9	2.06	100	11.5	1.41
10	0	33	2.04	100	9.7	1.17

[0306] **Example 8: In Vivo Assay for Combination of Compound (1) With Oseltamivir**

[0307] Infected mice were treated with vehicle or escalating dose levels of Form A of HCl salt of Compound (**1**)•1/2H_2O (hereinafter simply Compound (**1**) for Example 8) in combination with zanamivir starting 24 hours prior to influenza A challenge with 5×10^3

$TCID_{50}$ A/PR/8/34. The influenza A challenge and Compound **(1)** suspensions were prepared in a similar manner as described above in Example 7. The challenged mice were treated once, IN (intranasal), with zanamivir at 0.3 mg/kg, 1 mg/kg or 3 mg/kg 24hours prior to IN challenge with 5×10^3 $TCID_{50}$ A/PR/8/34, and with Compound (**1**) at 0.1 mg/kg, 0.3 mg/kg, or 1mg/kg BID for 10 days starting -2 hours prior to the challenge with 5×10^3 $TCID_{50}$ A/PR/8/34.

[0308] The results are summarized in Tables 19A and 19B below. As shown in Tables 18A below, the combination therapy with Compound (**1**) and zanamivir provided extra survival benefit (Table 19A). Efficiency quotient, a composite measure of survival, bodyweight loss and lung function (% survival/ (% body weight loss at Day 8)*(Penh at Day 6)) is summarized in Table 19B.

[0309] Table 19A: Survival Rate: Combination Therapy of Compound (**1**) with Zanamivir.

		Compound (1) (mg/kg, BID) 1st dose 2h prior to infection			
			0.1	**0.3**	**1**
Zanamivir (mg/kg, IN x 1), 1st dose 24 h prior to infection	**0**	0	12.5	44.4	100
	0.3	37.5	0	100	100
	1	50	75	100	100
	3	62.5	100	100	100

[0310] Table 19B: Efficiency Quotient: Combination Therapy of Compound (**1**) with Zanamivir.

		Compound (1) (mg/kg, BID) 1st dose 2h prior to infection			
			0.1	**0.3**	**1**
Zanamivir (mg/kg, IN x 1), 1st dose 24 h prior to infection	**0**	--	--	0.59	2.32
	0.3	0.44	--	1.35	2.97
	1	0.73	1.00	1.61	2.31
	3	0.73	1.30	1.48	4.28

[0311] Example 9: Prophylactic and Post-Infection Efficacy of Compound (1) in the Mouse Influenza A Infection Model

[0312] *Materials and Methods*

[0313] *Animals:* Female 18-20 g BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME) for the antiviral experiment. The animals were maintained on standard rodent chow and tap water *ad libitum*. They were quarantined for 48 hours prior to use.

[0314] *Virus:* Mouse-adapted Influenza A/California/04/2009 (pndH1N1) virus was obtained from Dr. Elena Govorkova (St. Jude Children's Research Hospital, Memphis, TN).

The virus stock was amplified in MDCK cells, followed by titration for lethality in BALB/c mice. Influenza A/Victoria/3/75 (H3N2) virus was obtained from the American Type Culture Collection (Manassas, VA). The virus was passaged seven times in mice to mouse-adapt it, followed one passage in MDCK cells. The virus was further titrated for lethality in BALB/c mice to obtain the proper lethal challenge dose. Influenza A/Vietnam/1203/2004 (H5N1) virus was obtained from Dr. Jackie Katz of Centers for Disease Control (Atlanta, GA). Mice were exposed to a lethal dose of the virus (5 MLD50, 5 PFU/mouse), which has previously resulted in death between days 6-13, with 90-100% mortality by day 10 at this dose.

[0315] *Compounds:* Oseltamivir (as Tamiflu®) was obtained from a local pharmacy. Each capsule of Tamiflu contains 75 mg of the active component, oseltamivir carboxylate, upon metabolism in the body. The dose of oseltamivir was based upon this measurement. Form A of HCl salt of Compound (**1**) hemihydrate (hereinafter simply Compound (**1**) for Example 9) was for the study and the dose of the compound was based upon the HCl salt of Compound (**1**) hemihydrate. Both Compound (**1**) and oseltamivir were prepared in 0.5% methylcellulose (Sigma, St. Louis, MO) for oral gavage (p.o.) administration to mice.

[0316] *Experiment design:* The mice were anesthetized by intraperitoneal injection of ketamine/xylazine (50/5 mg/kg), and the animals were infected intranasally with a 90-μl suspension of influenza virus. The virus challenge was approximately four 50% mouse lethal infectious doses. Treatments were given twice a day (at 12 hours intervals) for 10 days starting 2 hours before virus challenge or 48 hours post challenge as indicated. Parameters for assessing the infection were survival, mean day of death, body weight changes, and lung infection parameters (hemorrhage score, weight, and virus titer). Animals were weighed individually every other day through day 21 of the infection. Mice that died during the first six days of treatment period were deemed to have died from causes other than influenza virus infection, and were excluded from the total counts.

[0317] To assess lung infection parameters, lungs from sacrificed animals (initially 5 animals per group set apart for this purpose) were harvested. Lung hemorrhage score was assessed by visual inspection for color changes from pink to plum. This occurs regionally in the lungs, rather than by a gradual change of the whole lung to the darker color. Hemorrhage scores ranged from 0 (normal) to 4 (total lung showing plum color), and thus is a non-parametric measurement. The lungs were weighed and then frozen at -80 °C. Later, thawed lungs were homogenized in 1 ml of cell culture medium, the supernatant fluids were centrifuged to remove particulate matter, and the liquid samples were re-frozen at -80 °C. After preparing 96-well plates of MDCK cells, the samples were thawed, serially diluted in

10-fold dilution increments and titrated by endpoint dilution method in the plates (1), using 4 microwells per dilution. Virus titers were calculated as log10 50% cell culture infectious doses per gram of lung tissue (log10 CCID50/g).

[0318] *Statistical analysis*: Kaplan-Meir plots for multiple group comparisons were analyzed by the Mantel-Cox log-rank test to determine statistical significance. Subsequently, pairwise comparisons were made by the Gehan-Breslow-Wilcoxon test. The relative experimental significance was adjusted to a Bonferroni corrected significance threshold based on the number of treatment comparisons made. Mean day of death and mean lung hemorrhage score comparisons were analyzed by the Kruskal-Wallis test followed by Dunn's multiple comparisons test. Mean body weights, lung weights, and log10 lung virus titers were evaluated by ANOVA assuming equal variance and normal distribution. Following ANOVA, individual treatment values were compared by the Tukey-Kramer multiple comparisons test. Analyses were made using Prism® software (GraphPad Software, San Diego, CA).

[0319] *Results and Discussions*

[0320] The prophylactic dose response of Compound (**1**) was investigated in the mouse influenza A model. Dosing with vehicle or Compound (**1**) was initiated 2 h prior to infection and continued twice daily for 10 days. The results are summarized in Tables 20 and 21. All of the mice that received vehicle alone succumbed to the infection by study day 9 and had lost, on average, ~32% of their body weight (BW). Compound (**1**) administered at 1, 3 or 10 mg/kg BID provided complete survival and a dose-dependent reduction in BW loss. Compound (**1**) administered at 0.3 mg/kg BID provided some survival benefit (2/8 mice) although the mice had significant BW loss. In the same experiment, mice were dosed with oseltamivir at 10 mg/kg BID, a clinically-equivalent human dose (based on AUC). All of the oseltamivir-administered mice survived with a similar weight loss profile to mice administered 1 mg/kg BID Compound (**1**).

[0321] Compound (**1**) still provided effectiveness in this model challenged with Influenza A/Vietnam/1203/2004 (H5N1) virus when it was administered at 48 hours post infection, with continued BID dosing for 10 days (Table 22). Dosing of Compound (**1**) at 10 mg/kg provided complete protection as shown in Table 20.

[0322] Table 20: Effects of Prophylaxis with Compound (**1**) and Oseltamivir on an Influenza A/California/04/2009 (pndH1N1) Virus Infection in BALB/c mice (prophylaxis).

			Mean Lung Parameters (Day 6)		
Compound (mg/kg)[a]	**Survivors / Total**	**MDD[b] ± SD**	**Score**	**Weight (mg)**	**Virus Titer[c]**
Compound (**1**) (10 mg/kg)	10/10***	-	0.2 ± 0.4**	132 ± 20***	<2.6[d]***
Compound (**1**) (3 mg/kg)	9/9***	-	0.0 ± 0.0***	123 ± 21***	3.1 ± 0.9***
Compound (**1**) (1 mg/kg)	10/10***	-	0.6 ± 0.9[e]	246 ± 21*	5.5 ± 1.2***
Oseltamivir (10 mg/kg)	10/10***	-	1.0 ± 0.0[e]	178 ± 28***	7.9 ± 0.2
Placebo	2/20	9.9 ± 1.3	3.4 ± 0.5	282 ± 26	7.9 ± 0.4

[a] Dose per treatment, given twice a day for 10 days starting 2 hours prior to virus exposure.

[b] Mean day of death of mice that died on or before day 21.

[c] Log10 CCID50/g.

[d] Below limit of detection (2.6 log10).

[e] Not significant by the very stringent Dunn's multiple comparison test, but significant from placebo (P<0.01) by the pairwise two-tailed Mann-Whitney U-test. * P<0.05, ** P<0.01, *** P<0.001, compared to placebo.

[0323] Table 21: Effects of Compound (**1**) and Oseltamivir on an Influenza A/Victoria/3/75 (H3N2) Virus Infection in BALB/c mice (prophylaxis).

			Mean Lung Parameters (Day 6)		
Compound (mg/kg)[a]	**Survivors/ Total**	**MDD[b] ± SD**	**Score**	**Weight (mg)**	**Virus Titer[c]**
Compound (**1**) (10 mg/kg)	10/10***	-	0.1 ± 0.2[d]	164 ± 11**	6.1 ± 0.5***
Compound (**1**) (3 mg/kg)	10/10***	-	3.3 ± 0.6[e]	260 ± 25	7.2 ± 0.2
Compound (**1**) (1 mg/kg)	4/10	9.8 ± 1.9	3.2 ± 0.3[e]	274 ± 49	7.3 ± 0.3

			Mean Lung Parameters (Day 6)		
Compound (mg/kg)[a]	**Survivors/ Total**	**MDD[b] ± SD**	**Score**	**Weight (mg)**	**Virus Titer[c]**
Oseltamivir (10 mg/kg)	9/10***	7.0	1.7 ± 1.1	218 ± 24	7.0 ± 0.3**
Placebo	3/20	9.8 ± 2.1	2.2 ± 0.6	264 ± 54	7.8 ± 0.4

[a] Dose per treatment, given twice a day for 10 days starting 2 hours prior to virus exposure.

[b] Mean day of death of mice that died on or before day 21.

[c] Log10 CCID50/g.

[d] Not significant by the very stringent Dunn's multiple comparison test, but significant from placebo (P<0.01) by the pairwise two-tailed Mann-Whitney U-test.

[e] Same as footnote "d", but significant from placebo at P<0.05 level. ** P<0.01, *** P<0.001, compared to placebo.

[0324] Table 22: Effects of Treatment (+48h) with Compound (**1**) and Oseltamivir on an Influenza A/Vietnam/1203/2004 (H5N1) Virus Infection in BALB/c mice.

			Mean Lung Parameters (Day 6)	
Compound (mg/kg)[a]	**Survivors/ Total**	**MDD[b] ± SD**	**Weight (mg)**	**Virus Titer[c]**
Compound (**1**) (10 mg/kg)	10/10	>21	0.15 ± 0.02	3.75 ± 0.94
Oseltamivir (10 mg/kg)	0/10	9.5 ± 1.2	0.17 ± 0.02	5.22 ± 0.38
Placebo	0/20	9.9 ± 0.8	0.16 ± 0.02	4.65 ± 1.23

[a] Dose per treatment, given twice a day for 10 days starting 2 hours prior to virus exposure.

[b] Mean day of death of mice that died on or before day 21.

[c] Log10 CCID50/g.

[0325] Example 10: In Vitro Efficacy of Compound (1) Against A Span of Influenza Strains

[0326] *Cells and Viruses.* Madine Darby Canine Kidney (MDCK) cells were originally obtained from American Type Culture Collection (ATCC, Manassas, VA) and passaged

using standard laboratory techniques prior to use in infection assays. Cells were maintained at 37 °C in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), 2 mM L-glutamine, 10 mM HEPES, 100 U/mL penicillin and 100 ug/mL streptomycin (Invitrogen). Influenza virus was obtained from ATCC, the Virus Surveillance and Diagnosis Branch of the Influenza Division of the Centers for Disease Control and Prevention (CDC; Atlanta, GA) or the Influenza Reagent Resource, Influenza Division, WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, CDC. To generate viral stocks, MDCK cells were infected with a low multiplicity of infection (MOI) in DMEM supplemented with 2mM L-glutamine, 10mM HEPES, 100 U/mL penicillin, 100ug/mL streptomycin and 1 μg per mL tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin (USB Corp.; Santa Clara, CA). Cells were incubated at 37°C with 5% CO_2 for 48 h, after which time the supernatant was harvested by centrifugation at 900 × g for 10 min with a Beckman GS-6R centrifuge. Virus stocks were aliquoted and frozen at -80°C.

[0327] *Compounds.* Free base or HCl salt of Compound (**1**) (e.g., amorphous HCl salt of Compound (**1**), Form A of HCl salt of Compound (**1**) hemihydrate, amorphous free base Compound (**1**)) (hereinafter simply Compound (**1**) for Example 10) was dissolved in 100% dimethyl sulfoxide (DMSO) to make a solution of a concentration of 10 mM.

[0328] *Antiviral Activity.* The antiviral activity of Compound (**1**) was evaluated in MDCK cells as measured by ATP levels using CellTiter-Glo (Promega; Madison, WI). MDCK cells were plated into black, clear bottom, 384-well plates to a density of 2×10^4 cells per well in 50 μL VGM. Cells were incubated at 37 °C, 5% CO_2, in saturated humidity to allow cells to adhere and form a monolayer. After 5 h 40 μL of media was removed and 15 μL of virus was added at an MOI of 0.005. Compound was added as 25 μL of a ten point, three-fold dilution in DMEM with supplements (final DMSO concentration of 0.5%). Internal controls consisted of wells containing cells only and untreated cells infected with virus. After a 72 h incubation, 20 μL of CellTiter-Glo was added to each well and incubated at room temperature for 10 min. Luminescence was measured using an EnVision Multilabel reader (PerkinElmer; Waltham, MA). EC_{50} values (concentration of compound that ensures 50% cell viability of uninfected control) were calculated by fitting the compound dose versus response data using a 4-parameter curve fitting method employing a Levenburg Marquardt algorithm (Condoseo software; Genedata, Basel, Switzerland). *In vitro* testing of hpaiH5N1 was performed at Southern Research Institute under BSL-3 containment.

[0329] As shown in Table 23 below, Compound (1) showed potent activity against all influenza A strains tested, including H1N1 and H3N2 reference strains from 1934 to 2009, as well as the pandemic 2009 H1N1 strains A/California/07/2009, A/Texas/48/2009, and the highly pathogenic avian H5N1 strain A/VN/1203/2004. Compound (1) was equally effective against all strains including those that were resistant to amantadine and neuraminidase inhibitors. It showed limited activity against influenza B virus.

[0330] Table 23: Efficacy of Compound (1) Against a Panel of Influenza Strains

Influenza Strain	Inf. Virus Strain	Subtype	Cell Protection Assay[e] $EC_{50} \pm SD$
			Comp (1) (nM)
A/WS/33[a]	A	H1N1	3.2 ± 4.3
A/NWS/33[a]	A	H1N1	0.73 ± 0.10
A/Puerto Rico/8/34[a]	A	H1N1	3.2 ± 1.8
A/Weiss/43[a]	A	H1N1	0.31 ± 0.23
A/FM/1/47	A	H1N1	0.57 ± 0.036
A/Mal/302/54	A	H1N1	0.57 ± 0.055
A/Denver/1/57	A	H1N1	0.42 ± 0.19
A/Chelyabinsk/1/2006	A	H1N1	0.70 ± 0.49
A/Florida/3/2006	A	H1N1	0.92 ± 1.5
A/Fukushima/141/2006	A	H1N1	0.18 ± 0.20
A/Georgia/17/2006	A	H1N1	0.13 ± 0.048
A/Georgia/20/2006[b]	A	H1N1	2.6 ± 3.8
A/Missouri/3/2006	A	H1N1	0.21 ± 0.060
A/St. Petersburg/8/2006[a]	A	H1N1	0.88 ± 0.69
A/Virginia/01/2006[a]	A	H1N1	0.42 ± 0.24
A/Cambodia/0371/2007[a*]	A	H1N1	0.61 ± 0.33
A/South Dakota/6/2007	A	H1N1	0.31 ± 0.25
A/California/07/2009 NYMC X-179A[a]	A	H1N1	2.7 ± 1.8
A/Aichi/2/68	A	H3N2	1.4 ± 1.1
A/Hong Kong/8/68	A	H3N2	0.60 ± 0.11
A/Port Chalmers/1/73[a]	A	H3N2	0.54 ± 0.11
A/Victoria/3/75	A	H3N2	1.3 ± 0.63
A/Wisconsin/67/2005[a]	A	H3N2	1.8 ± 0.24
A/Hawaii/2/2006	A	H3N2	1.4 ± 0.91
A/Nebraska/1/2006[a*]	A	H3N2	2.1 ± 1.3
A/Texas/12/2007[a*c]	A	H3N2	0.65 ± 0.22
A/Uruguay/716/2007[a]	A	H3N2	3.5 ± 5.1
A/New Jersey/8/76	B	H1N1	0.20 ± 0.096
A/California/07/2009[a]	C	H1N1	1.8 ± 1.6
A/Mexico/4108/2009[a]	C	H1N1	2.7 ± 1.8
A/New York/18/2009[a*]	C	H1N1	0.59 ± 0.40
A/Texas/48/2009[b]	C	H1N1	2.8 ± 3.2
A/Virginia/ATCC2/2009	C	H1N1	1.9 ± 3.0
A/Virginia/ATCC3/2009	C	H1N1	1.9 ± 3.2

Influenza Strain	Inf. Virus Strain	Subtype	Cell Protection Assay[e] $EC_{50} \pm SD$
			Comp (1) (nM)
A/Swine/Iowa/15/30	C	H1N1	0.65 ± 0.082
A/Swine/1976/31	C	H1N1	0.47 ± 0.11
A/Equine/2/Miami/63	C	H3N8	0.50 ± 0.065
A/Viet Nam/1203/2004[a]	K	H5N1	<1.5 ± ND
B/Lee/40			>10 ± ND
B/Russia/69			>10 ± ND

[a]: amantadine resistance: M2 31N mutation.

[b]: oseltamivir carboxylate resistance: NA 275Y mutation.

[c]: oseltamivir carboxylate resistance: NA 119V mutation.

[*]: externally validated phenotypic resistance, sequence data unavailable.

[0331] Example 11: In Vitro Combination Experiments with Compound (1) and Oseltamivir, Zanamivir, or Favipiravir

[0332] A solution of Compound (**1**) (free base or HCl salt of Compound (**1**) similarly in Example 10) in 100% dimethyl sulfoxide (DMSO) was tested in a three day MDCK cell CPE-based assay, infected with A/Puerto Rico/8/34 at an MOI of 0.01, in combination experiments with either the neuraminidase inhibitors oseltamivir carboxylate and zanamivir, or the polymerase inhibitor T-705. Oseltamivir carboxylate and T-705 were dissolved in 100% dimethyl sulfoxide (DMSO); zanamivir was dissolved in Dulbecco's modified eagle medium (DMEM) at a concentration of 10 mM and stored at -20°C. The study employed either the Bliss independence method (Macsynergy) (e.g., Prichard, M.N. and C. Shipman, Jr., *Antiviral Res*, 1990. **14**(4-5): p. 181-205) or the Loewe additivity/Median-effect method (e.g., Chou, T.C. and P. Talalay, *Adv Enzyme Regul*, 1984. **22**: p. 27-55). The Bliss independence method involves testing different concentration combinations of inhibitors in a checkerboard fashion, while the Loewe independence method involves testing a fixed ratio combination of inhibitors, at different dilutions of the fixed ratio. Experiments were also performed using combinations of Compound (**1**) with itself as a control, confirming additivity. Cell viability was determined using CellTiter-Glo.

[0333] The Bliss independence method resulted in synergy volumes of 312 and 268 for oseltamivir carboxylate and zanamivir, respectively; and a synergy volume of 317 was obtained for favipiravir. Synergy volumes greater than 100 are generally considered strong synergy and volumes between 50 and 100 are considered moderate synergy. The Loewe

additivity method produced C.I. (combination index) values of 0.58, 0.64, and 0.89 at the 50% effect level for oseltamivir, zanamivir, and T-705, respectively. C.I. values of less than 0.8 are considered strong synergy while values between 0.8 and 1.0 are considered additive to mildly synergistic. These data together, as shown in Table 24, suggest that Compound (**1**) is synergistic with the neuraminidase inhibitors and polymerase inhibitor tested.

[0334] Table 24: Summary of In Vitro Synergy and Antagonism Experiments

Loewe Additivity	**Combination Index**			**Result**
	ED_{50}	**ED_{75}**	**ED_{90}**	
Compound (**1**) + oseltamivir	0.60, 0.56	0.57, 0.56	0.59, 0.58	Strong synergy
Compound (**1**) + zanamivir	0.68, 0.61	0.67, 0.66	0.71, 0.77	Strong synergy
Compound (**1**) + favipiravir	0.83, 0.96	0.76, 1.0	0.71, 1.1	Additivity to weak synergy
Bliss Independence	**Synergy Volume, 95% Confidence**			**Result**
Compound (**1**) + oseltamivir	312			Strong synergy
Compound (**1**) + zanamivir	268			Strong synergy
Compound (**1**) + favipiravir	317			Strong synergy

ED_{50}, ED_{75}, ED_{90}: Compound concentration at which 50%, 75%, or 90%, respectively, of cells are Protected; Combination indexes were calculated at the effect levels of ED_{50}, ED_{75} and ED_{90}.

[0335] Example 12: Efficacy in the Mouse Influenza A Infection Model

[0336] The prophylactic dose response of Compound (**1**) (in amorphous or Form A of HCl salt of Compound (**1**) hemihydrate (hereinafter in this example simply Compound (**1**)) was investigated in the mouse influenza A model. Dosing with vehicle or Compound (**1**) was initiated 2 h prior to infection and continued twice daily for 10 days. All of the mice that received vehicle alone succumbed to the infection by study day 9 and had lost, on average, ~32% of their body weight (BW). Compound (**1**) administered at 1, 3 or 10 mg/kg BID provided complete survival and a dose-dependent reduction in BW loss. Compound (**1**) administered at 0.3 mg/kg BID provided some survival benefit (2/8 mice) although the mice had significant BW loss. In the same experiment, mice were dosed with oseltamivir at 10 mg/kg BID, a clinically-equivalent human dose (based on AUC). All of the oseltamivir-administered mice survived with a similar weight loss profile to mice administered 1 mg/kg BID Compound (**1**).

[0337] The extent to which Compound (**1**) administration could be delayed and still provide effectiveness in this model was investigated by challenging mice with influenza A virus and dosing with vehicle, oseltamivir, or Compound (**1**) starting at 24, 48, 72, 96 or 120 h post

infection, with continued BID dosing for 10 days (Table 25). All vehicle controls succumbed to disease by study days 8 or 9. Compound (**1**) administered at 1, 3 or 10 mg/kg BID provided complete protection from death and reduced BW loss when dosing was initiated up to 72 h post infection compared with vehicle controls. Dosing of oseltamivir at 10 mg/kg BID only provided complete protection when dosing was initiated 24 h or less, post infection. When initiation of compound administration was delayed further, Compound (**1**) at 3 or 10 mg/kg BID provided complete survival at 96 h post infection and partial protection when initiation of dosing was delayed 120 h post infection.

[0338] The effectiveness of Compound (**1**) to reduce lung viral titers was investigated. Mice were infected with influenza A and 24 h later vehicle, oseltamivir (10 mg/kg BID) or Compound (**1**) (3, 10, 30 mg/kg BID) was administered until lung harvest and viral burden determination on day 6 (Table 26). All Compound (**1**)-administered groups showed robust, statistically significant reductions in lung viral titers compared with oseltamivir- and vehicle-administered animals.

[0339] In order to establish a PK/PD model, mice were infected with influenza virus for 24 h and then administered Compound (**1**) for an additional 24 h. Doses were fractionated as a single dose, two or four doses administered every 12 h or 6 h, respectively. Lungs and plasma were collected to determine lung viral loads and Compound (**1**) concentrations. The individual lung titer data from these dosing regimens (q6h, q12h and q24h) was plotted against individual C_{max} C_{min} or AUC values (data not shown). While there was a clear correlation between lung titer reduction and C_{min}, there was little correlation with C_{max} and only a weak correlation with AUC. There was a strong correlation with C_{min} when the measured Compound (**1**) concentrations in plasma was plotted versus the measured lung titers. The half maximal reduction in lung titers (2-3 log) occurs near the serum-shifted EC_{99} (100 ng/mL). A similar correlation was found between lung titer and measured Compound (**1**) concentrations in the lungs (data not shown).

[0340] Table 25: Summary of Percent Survival and Percent Body Weight Loss in Mouse Model of Influenza A.

Treatment Start Time Relative Infection (h)	**Compound (1) Dose (mg/kg; BID)**	**Oseltamivir Dose (mg/kg; BID)**	**Percent Survival**	**Percent Body Weight Loss on Study Day 8**
-2[a]	10		100	-2.8
	3		100	-8.7
	1		100	-16.8
	0.3		25	-30.4

Treatment Start Time Relative Infection (h)	Compound (1) Dose (mg/kg; BID)	Oseltamivir Dose (mg/kg; BID)	Percent Survival	Percent Body Weight Loss on Study Day 8
	0.1		0	-31.9
		10	100	-19.1
	0		0	-32.2
+24[a]	10		100	-6.2
	3		100	-14.2
	1		100	-23.4
		10	100	-28.9
	0		0	-33.8
+48[a]	10		100	-7.1
	3		100	-10.9
	1		100	-22.5
		10	80	-31.1
	0		0	-34.4
+72[a]	10		100	-17.4
	3		100	-23.2
	1		100	-29.4
		10	0	-31.3
	0		0	-36.1
+96[b]	10		100	-25.5
	3		100	-27.3
		10	ND[c]	ND[c]
	0		0	-34.6
+120[b]	10		37.5	-34.4
	3		12.5	-32.6
		10	ND[c]	ND[c]
	0		0	-34.6

[a] Data are from independent experiments.

[b] Data are from the same experiment.

[c] ND, not determined.

[0341] Table 26: Summary of Lung Viral Titer and Log_{10} Reduction in Mouse Model of Influenza A.

Treatment[a]	Study 1		Study 2	
	Lung Viral Titer (Log_{10} $TCID_{50}$)[b]	Log_{10} Reduction vs. Vehicle	Lung Viral Titer (Log_{10} $TCID_{50}$)[b]	Log_{10} Reduction vs. Vehicle
10 mg/kg BID Vehicle	6.20		6.28	
10 mg/kg BID Oseltamivir	6.05	-0.15		
30 mg/kg BID Compound (1)	3.95	-2.25***	4.53***	-1.75
10 mg/kg BID Compound (1)			5.20***	-1.08

Treatment[a]	Study 1		Study 2	
	Lung Viral Titer (Log_{10} $TCID_{50}$)[b]	Log_{10} Reduction vs. Vehicle	Lung Viral Titer (Log_{10} $TCID_{50}$)[b]	Log_{10} Reduction vs. Vehicle
3 mg/kg BID Compound (**1**)			5.24***	-1.04

[a] Animal Treatment was initiated 24 houses post infection and continued for 5 days.

[b] Lung viral titers were determined on study day 6.

[c] ND, not determined.

2 way ANOVA with Bonferroni Post Test, ***P<0.001.

[0342] Example 13: Proof-of-Concept Influenza Challenge

[0343] A live, attenuated influenza challenge model was used previously to predict the effectiveness of influenza antivirals in natural infection in humans (Calfee, D.P., Peng, A.W., Hussey, E.K., Lobo, M. & Hayden F.G. Safety and efficacy of once daily intranasal zanamivir in preventing experimental human influenza A infection. *Antivir Ther.* **4**, 143-149 (1999); Hayden, F.G. *et al.* Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza. *JAMA* **282**, 1240-1246 (1999)). A randomized, double-blinded, placebo-controlled, single center study of Form A of HCl salt of Compound (**1**) hemihydrate (hereinafter in this example simply Compound (**1**)) in healthy volunteers inoculated with live influenza A/Wisconsin/67/2005 (H3N2) challenge strain virus was conducted. Subjects received five daily doses of either placebo (N=33) or Compound (**1**) once a day (QD) (in capsule form consisting of neat Compound (**1**)): 100 mg (N=16), 400 mg (N=19), or 900 mg on Day 1 followed by 600 mg Days 2–5 (N=20), or 1200 mg on Day 1 followed by 600 mg Days 2–5 (N=18). Subjects underwent thrice daily nasal swabs, and kept thrice daily score cards for clinical symptoms from Days 1-7, and were discharged from the facility on Day 8, with safety follow-up at approximately Day 28. Nasal swabs were assayed for influenza virus in cell culture (primary analysis) and by qRT-PCR (secondary analysis).

[0344] Efficacy analyses were performed on the Full Analysis (FA) Set, defined as all randomized subjects who received at least one dose of study drug (Compound (**1**) or placebo) and whose viral concentrations were above or equal to the lower limit of quantification for the $TCID_{50}$ cell culture assay at any time point within 48 h post inoculation, or whose hemagglutination inhibition titer raised 4-fold or greater from baseline (Day 1) in the post inoculation period (N=74). The safety set included all subjects who were inoculated with influenza on Day 0 and who received at least one dose of either placebo or Compound (**1**) (N=104).

[0345] *Efficacy Assessment*

[0346] The primary measure in this study was demonstration of a dose response trend in AUC of viral shedding between study Days 1 (first day of drug dosing) through 7, as measured by $TCID_{50}$ in cell culture assay in the FA set. A statistically significant dose response trend was observed in median AUC viral shedding in nasal swabs (P=0.036, Jonckheere-Terpstra trend test). In addition, pairwise comparisons were performed between the pooled placebo group and each Compound (**1**) dose group for median AUC viral shedding, median duration of shedding, and mean magnitude of peak viral shedding (Table 27). A statistically significant reduction in AUC viral shedding was observed for the 1200/600 mg dose group (P=0.010, Wilcoxon rank-sum test), and significant reductions in peak shedding were observed for the 1200/600 mg dose group (FIG. 13), the 400 mg dose group and the pooled Compound (**1**) dose groups. Additional FA group analyses were performed (data not shown).

[0347] Nasal influenza shedding was also quantified by qRT-PCR and results were similar to those observed with cell culture. There was no difference in rates of seroconversion between Compound (**1**) dose groups and placebo, as defined by a 4-fold or greater increase in anti-influenza titer from pre-inoculation baseline, suggesting that Compound (**1**) dosed 24 h after influenza inoculation did not affect the rate of acquisition of influenza infection and did not eliminate the subsequent humoral immune response to infection (Table 28).

[0348] Subjects recorded clinical symptoms three times a day in diaries. An AUC of clinical and influenza-like symptom scores from Day 1 through Day 7 was calculated. Compared with placebo, the 1200/600 mg dose group of Compound (**1**) showed a statistically significant reduction in the median duration of composite clinical symptoms (P=0.001), the median AUC of influenza-like symptoms (P=0.040), and the median duration of influenza-like symptoms (P<0.001) (Table 28).

[0349] Table 28: Median AUC viral shedding, median duration of shedding, and mean magnitude of peak viral shedding.

Endpoint [units]		**Pooled Placebo (N=22)**	**Compound (1)**				
			100 mg (N=12)	**400 mg (N=12)**	**900/600 mg (N=14)**	**1200/600 mg (N=14)**	**Pooled (N=52)**
Viral Shedding by Tissue Culture[a]	AUC, median (range)	5.85	1.25	0.70	3.20	0.35	0.65
	[$\log_{10}$ $TCID_{50}$	(0.0, 17.1)	(0.0, 16.1)	(0.0, 18.0)	(0.0,16.1)	(0.0,8.4)	(0.0, 18.0)

Endpoint [units]		Pooled Placebo (N=22)	Compound (1)				
			100 mg (N=12)	**400 mg (N=12)**	**900/600 mg (N=14)**	**1200/600 mg (N=14)**	**Pooled (N=52)**
	mL*Day]						
	P Value[b]	NA	0.269	0.206	0.723	**0.010**	0.057
	Duration , median	2.38	0.96	1.60	2.71	0.00	0.71
	(95%CI) [Day]	(0.03, 4.63)	(0.00, 3.39)	(0.00, NA)	(0.00,4.68)	(0.00,1.33)	(0.00, 2.43)
	P Value[d]	NA	0.331	0.831	0.893	0.169	0.487
	Peak, mean (SD)	3.13	2.09	1.73	2.68	1.00	1.87
	[$\log_{10}$ $TCID_{50}$/ mL]	(1.878)	(2.209)	(1.976)	(2.201)	(1.365)	(2.002)
	P Value[c]	NA	0.139	**0.049**	0.505	**0.002**	**0.015**
Viral Shedding by qRT-PCR[e]	AUC, median (range)	18.40	6.05	4.90	10.65	0.45	3.45
	[$\log_{10}$ copies/m L*Day]	(0.0, 42.1)	(0.0, 41.9)	(0.0, 36.9)	(0.0,37.1)	(0.0,24.7)	(0.0, 41.9)
	P Value[b]	NA	0.218	0.306	0.821	**0.014**	0.075
	Duration , median	2.91	0.96	1.36	2.39	0.00	0.71
	(95%CI) [Day]	(0.03, 5.35)	(0.00, 3.39)	(0.00, NA)	(0.00,5.01)	(0.00,0.66)	(0.00, 2.394)
	P Value[d]	NA	0.318	0.753	0.602	0.084	0.238
	Peak, mean (SD)	5.36	4.36	3.90	5.08	2.37	3.91
	[$\log_{10}$ $TCID_{50}$/ mL]	(3.108)	(3.379)	(3.514)	(3.097)	(2.861)	(3.276)
	P Value[c]	NA	0.380	0.202	0.794	**0.007**	0.081
Serology[f]	Sero-conversi on	21/32	11/16	9/19	13/19	12/18	45/72
	n/N (%)	(66%)	(69%)	(47%)	(68%)	(67%)	(63%)
	P Value	NA	>0.999	0.247	>0.999	>0.999	0.828

AUC: area under the value versus time curve; CI: confidence interval; NA: not applicable; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; SD: standard deviation; TCID50: 50% tissue culture infective dose.

Note: Statistically significant *P* values ($P<0.05$) are in bold font.

[a] P=0.036 for the dose response trend of AUC from Jonckheere-Terpstra trend test.

[b] P value calculated from Wilcoxon rank-sum test.

[c] Pvalue calculated from ANOVA.

[d] P value calculated from log-rank test.

[e] P = 0.031 for the dose response trend of AUC from Jonckherre-Terpstra trend test.

[f] Sero-conversion defined as ≥4-fold increase in anti-influenza antibody titer at Follow-up Visit compared with baseline. P value calculated using Fisher's Exact Test.

[0350] Table 28: Median AUC, median duration, and mean magnitude of peak, of composite clinical symptom and influenza like symptom.

Endpoint [units]		**Pooled Placebo (N=22)**	**Compound (1)**				
			100 mg (N=12)	**400 mg (N=12)**	**900/600 mg (N=14)**	**1200/600 mg (N=14)**	**Pooled (N=52)**
Composite Clinical Symptom	AUC, median (range)	4.85	1.85	4.70	1.75	1.95	2.15
	[Grade* Day]	(0.0, 23.5)	(0.0, 25.3)	(0.0, 16.0)	(0.0,32.3)	(0.0,5.5)	(0.0, 32.3)
	P Value[b]	NA	0.422	0.694	0.595	0.83	0.211
	Duration , median	3.69	3.21	3.34	2.69	1.88	2.34
	(95%CI) [Day]	(2.04, 4.73)	(0.03, 5.43)	(1.28, 4.63)	(0.00,4.61)	(0.00, 2.24)	(1.87, 3.06)
	P Value[d]	NA	0.946	0.994	0.686	**0.001**	0.355
	Peak, mean (SD)	3.91	3.17	2.83	3.71	1.50	2.79
	[Grade]	(3.637)	(3.881)	(2.167)	(4.232)	(1.286)	(3.158)
	P Value[c]	NA	0.532	0.366	0.863	**0.036**	0.187
Influenza like Symptom	AUC, median (range)	4.05	1.85	3.80	1.75	1.75	2.05
	[Grade* Day]	(0.0, 17.7)	(0.0, 21.3)	(0.0, 14.0)	(0.0,28.6)	(0.0,4.4)	(0.0, 28.6)
	P Value[b]	NA	0.363	0.617	0.595	**0.040**	0.149
	Duration , median	3.69	3.21	3.34	2.69	1.88	2.34
	(95%CI) [Day]	(2.04, 4.73)	(0.00, 5.40)	(1.28, 4.63)	(0.00,4.61)	(0.00,2.24)	(1.87, 3.00)
	P Value[d]	NA	0.957	0.994	0.653	**<0.001**	0.342
	Peak, mean (SD)	3.41	2.75	2.42	3.21	1.36	2.42

Endpoint [units]		Pooled Placebo (N=22)	Compound (1)				
			100 mg (N=12)	400 mg (N=12)	900/600 mg (N=14)	1200/600 mg (N=14)	Pooled (N=52)
	[Grade]	(3.003)	(3.361)	(1.832)	(3.534)	(1.216)	(2.689)
	P Value[c]	NA	0.511	0.323	0.838	**0.034**	0.168

AUC: area under the value versus time curve; CI: confidence interval; NA: not applicable.

Note: Statistically significant *P* values ($P<0.05$) are in bold font.

[b] *P* value calculated from Wilcoxon rank-sum test.

[c] *P*value calculated from ANOVA.

[d] *P* value calculated from log-rank test.

[0351] *Safety Assessment*

[0352] Compound (**1**) was well tolerated, and there were no discontinuations due to Compound (**1**)-related adverse events (AE) nor were there any serious adverse events. A list of adverse events occurring in ≥10% of subjects in any treatment group is presented (Table 29). Influenza-like illness was the most frequently reported adverse event, and was reported by an approximately equal proportion of subjects in the placebo and Compound (**1**) groups. Adverse events that occurred with ≥10% difference in incidence between the Compound (**1**) groups and the placebo recipients were: decreased blood phosphorus level (18.1%, Compound (**1**); 0%, placebo), rhinorrhea (Compound (**1**), 4.2%; 18.8%, placebo), and nasal congestion (1.4%, Compound (**1**); 15.6% placebo). In addition, elevations in alanine aminotransferase (ALT) were observed in both placebo and Compound (**1**) recipients. Neither liver function abnormalities nor serum phosphate decreases were observed in the first-in-human dose escalation study of Compound (**1**) at single doses up to 1600 mg and multiple doses up to 800 mg daily for 10 days; both elevations in ALT and decreases in serum phosphate have been previously reported with upper respiratory viral infections.

[0353] Table 29: A list of adverse events occurring in ≥10% of subjects in any treatment group

Preferred Term	Pooled Placebo	Compound (1)				
		100 mg	400 mg	900/600 mg[a]	1200/600 mg[b]	Pooled
	N=32 n(%)	N=16 n(%)	N=19 n(%)	N=19 n(%)	N=18 n(%)	N=72 n(%)
Influenza-like illness[c]	12 (37.5)	8 (50.0)	10 (52.6)	9 (47.4)	7 (38.9)	34 (47.2)
Alanine aminotransferase increased	5 (15.6)	3 (18.8)	1 (5.3)	0	6 (33.3)	10 (13.9)

Preferred Term	Pooled Placebo	Compound (1)				
		100 mg	400 mg	900/600 mg[a]	1200/600 mg[b]	Pooled
	N=32 n(%)	N=16 n(%)	N=19 n(%)	N=19 n(%)	N=18 n(%)	N=72 n(%)
Blood phosphorus decreased	0	3 (18.8)	0	6 (31.6)	4 (22.2)	13 (18.1)
Spirometry abnormal	2 (6.3)	2 (12.5)	4 (21.1)	0	4 (22.2)	10 (13.9)
Rhinorrhea	6 (18.8)	0	2 (10.5)	0	1 (5.6)	3 (4.2)
Headache	2 (6.3)	1 (6.3)	4 (21.1)	0	2 (11.1)	7 (9.7)
Dermatitis contact	3 (9.4)	3 (18.8)	0	0	0	3 (4.2)
Nasal congestion	5 (15.6)	0	0	0	1 (5.6)	1 (1.4)
Aspartate aminotransferase increased	1 (3.1)	1 (6.3)	1 (5.3)	0	2 (11.1)	4 (5.6)
Oropharylngeal pain	1 (3.1)	2 (12.5)	0	1 (5.3)	0	3 (4.2)
Tension Headache	1 (3.1)	0	2 (10.5)	1 (5.3)	0	3 (4.2)
Malaise	1 (3.1)	2 (12.5)	0	0	0	2 (2.8)
Nausea	0	0	2 (10.5)	1 (5.3)	0	3 (4.2)

Notes: A subject with multiple events was counted once under the AE. Subjects may appear in multiple categories.

[a] Single loading dose of 900 mg on Day 1and 600 mg qd on Days 2 through 5.

[b] Single loading dose of 1200 mg on Day 1 and 600 mg qd on Days 2 through 5.

[c] Influenza-like illness, as defined in the efficacy analysis, was assessed based on the parameters listed in the text. The AE of influenza-like illness was determined by physician.

[0354] *Discussion*

[0355] In an influenza challenge study in healthy volunteers, Compound (**1**) demonstrated a dose response trend in AUC viral titer in nasal swabs by both $TCID_{50}$ cell culture and qRT-PCR, and the highest dose of Compound (**1**) evaluated caused a significant reduction in AUC viral titer as well as in AUC and duration of influenza symptoms. Although, a similar magnitude of improvement over placebo was not observed in the second highest dose group, 900/600 mg (Table 27), this dose did demonstrate similar results to the 1200/600 mg dose with respect to median AUC for composite clinical symptom and influenza-like symptom endpoints (Table 28); the reasons for this discrepancy are not completely understood. While no definite safety trends were encountered in the POC trial, the phosphate decreases and ALT

elevations observed suggest that appropriate monitoring of both parameters will need to be employed in future studies.

[0356] Overall, the limitations of the influenza challenge model are that the influenza virus utilized in this study is a strain that has been specifically selected so as not to produce the most severe clinical symptoms of influenza virus infection. In addition, the viral inoculum administered is likely larger than the inoculum in natural influenza exposure. The timing of Compound (**1**) dosing 24 h after exposure may not be a realistic timeframe for initiation of therapy in the community setting in which patients do not often seek diagnosis or treatment until they have developed substantial symptoms, likely more than 24 h after exposure. However, given that naturally infected subjects are initially inoculated with a much lower viral titer the time scales are not directly comparable.

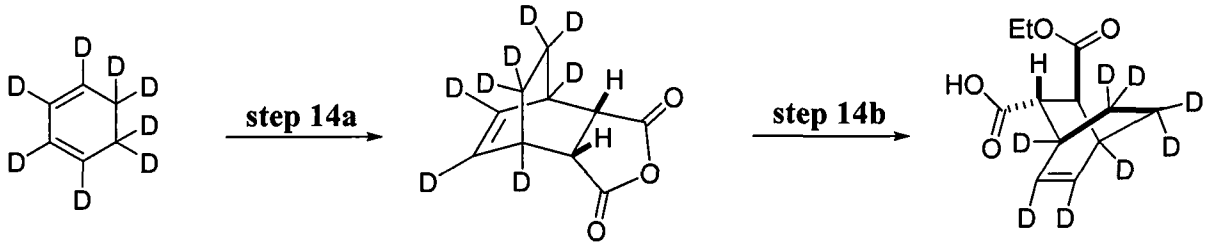
[0357] In summary, Compound (**1**) is a potent influenza A PB2 inhibitor that represents a distinct and novel class of antiviral agent. The properties of this inhibitor, as described by both the preclinical and clinical data, indicate that Compound (**1**) is an exciting candidate for further evaluation with several potential advantages over current antiviral agents used to treat influenza infection.

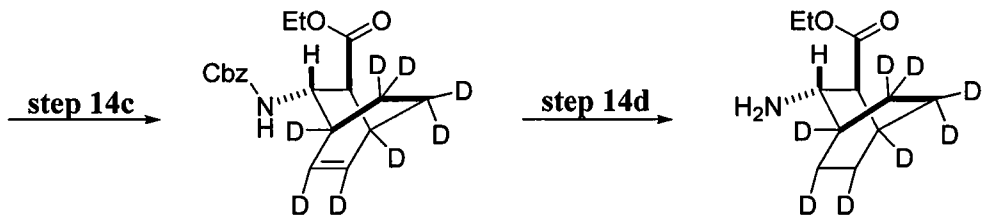
[0358] All references provided herein are incorporated in its entirety by reference. As used herein, all abbreviations, symbols and conventions are consistent with those used in the contemporary scientific literature. See, e.g., Janet S. Dodd, ed., *The ACS Style Guide: A Manual for Authors and Editors*, 2nd Ed., Washington, D.C.: American Chemical Society, 1997.

[0359] Example 14: Deuterium Enriched Compound (1).

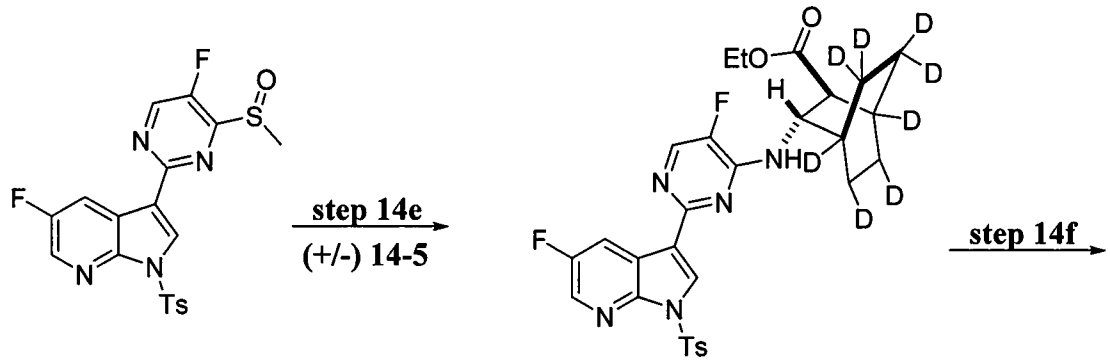
[0360] Deuterium enriched Compound (**1**) was synthesized according to Scheme 1, below:

[0361] Scheme 1:

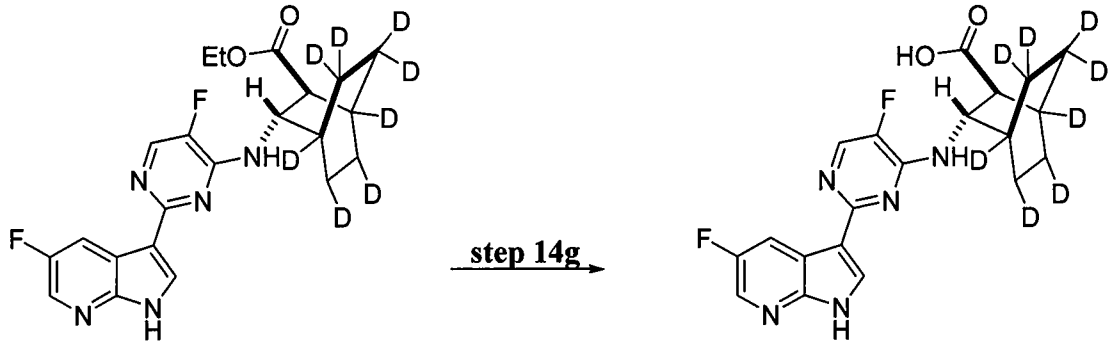




(+/-) 14-4 (+/-) 14-5



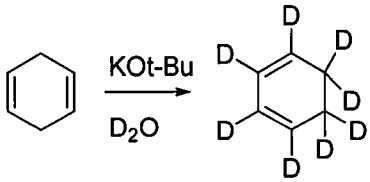
(+/-) 14-6 (+/-) 14-7



(+/-) 14-8 (+/-) (1)

[0362] Reagents and conditions: (step 14a) maleic anhydride, $CHCl_3$; (step 14b) beta-quinine, ethanol, toluene; (step 14c) DPPA, Et_3N, 90 °C, BnOH; (step 14d) H_2, Pd/C, MeOH; (step 14e) amine **14-5**, $^iPr_2NEt$, THF, 70 °C; (step 14f) HCl, dioxane, MeCN, 80 °C; (step 14g) NaOH, THF, MeOH.

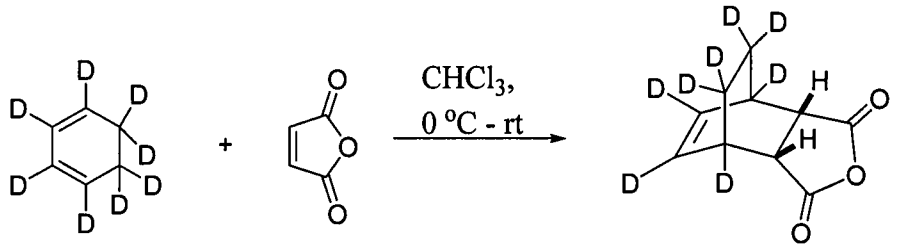
[0363] *14A: Compound (14-1)*



14-1

[0364] Potassium tert-butoxide (9.663 g, 86.11 mmol) was dissolved in DMSO-d_6 (30.00 mL) and placed under nitrogen. A solution of cyclohexa-1,4-diene (6 g, 74.88 mmol) in pentane (60.00 mL) was added and the mixture was stirred under nitrogen for 2.5 hrs. The DMSO-d_6 layer was removed, and a fresh 30 mL DMSO-d_6 with potassium tert-butoxide (9.663 g, 86.11 mmol) were added. Stirring was continued overnight. The layers were separated, and the pentane layer was washed with D_2O (50mL) and dried on Na_2SO_4 to generate 1,2,3,4,5,5,6,6-octadeuteriocyclohexa-1,3-diene (**14-1**), which was moved on to the next step as a solution. This reaction creates a mixture of 1,3- and 1,4-diene isomers. Only the 1,3-diene reacts in the subsequent step.

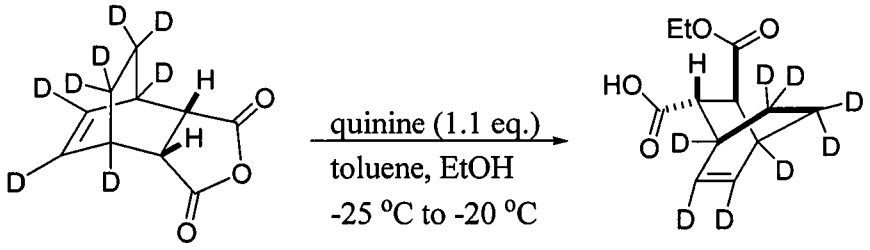
[0365] *14b: 3a,4,7,7a-tetrahydro-4,7-ethanoisobenzofuran-1,3-dione-4,5,6,7,8,8,9,9-d_8 (**14-2**)*



14-1 **14-2**

[0366] The pentane solution of 1,2,3,4,5,5,6,6-octadeuteriocyclohexa-1,3-diene (**14-1**) (6.5 g, 74.0 mmol) was diluted with chloroform (50 mL) and treated with maleic anhydride (8.0 g, 81.4 mmol). The reaction mixture was allowed to stir at room temperature overnight. The solvent was evaporated under reduced pressure and the resulting semi-solid residue was treated with MeOH. After stirring for 10 minutes, the MeOH slurry was cooled to approximately 20 °C. The resulting precipitate was collected by filtration and washed with three small (5mL) portions of cold methanol to provide the product (**14-2**) as a white solid: ^{1}H NMR analysis ($CDCl_3$) 3.15 (s, 2H) shows clean product and 95% deuterium incorporation.

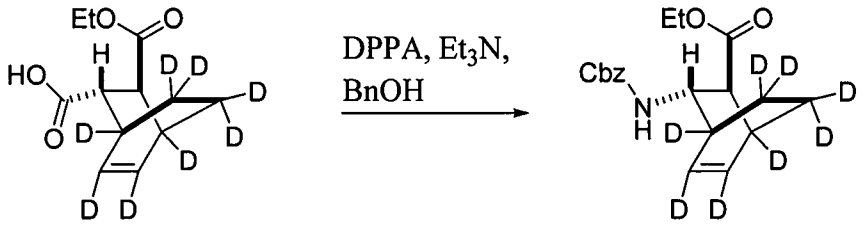
[0367] *14c: (+/-)-trans-3-(ethoxycarbonyl)bicyclo[2.2.2]oct-5-ene-2-carboxylic-1,4,5,6,7,7,8,8-d_8 acid (**14-3**)*



14-2 **(+/-) 14-3**

[0368] To a 3-neck RBF under nitrogen was attached an addition funnel and an internal temperature probe. The flask was charged with 3a,4,7,7a-tetrahydro-4,7-ethanoisobenzofuran-1,3-dione-4,5,6,7,8,8,9,9-d_8 (**14-2**) (2.68 g, 14.39 mmol), beta-quinine (5.24 g, 15.83 mmol) and anhydrous toluene (40 mL). The reaction was magnetically stirred and cooled to -25 °C (cold finger cooling). A solution of anhydrous absolute ethanol (8.40 mL, 143.90 mmol) in anhydrous toluene (13.4 mL) was added over 25 minutes maintaining an internal temperature below -25 °C. The reaction mixture was stirred at approximately -20 °C overnight. The precipitated gel-like solid was collected by filtration, washed with toluene (3×30 mL) and then taken up in aq. 1N HCl/EtOAc (300 mL of 1:1 mixture). The biphasic mixture was stirred until all precipitate dissolved. The layers were separated and the organic layer was washed with water (2×100mL), brine (100 mL), dried over Na_2SO_4, filtered, concentrated on the rotavaporator at low temperature to afford 800 mg of the desired product (**14-3**), which was used without further purification.

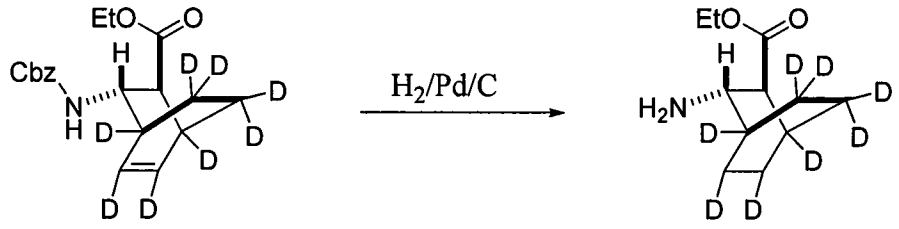
[0369] *14d:* (+/-)-*trans*-ethyl-3-(((benzyloxy)carbonyl)amino)bicyclo[2.2.2]oct-5-ene-2-carboxylate-1,4,5,6,7,7,8,8-d_8 ***(14-4)***



(+/-) 14-3 **(+/-) 14-4**

[0370] To a solution of (+/-)-*trans*-3-(ethoxycarbonyl)bicyclo[2.2.2]oct-5-ene-2-carboxylic-1,4,5,6,7,7,8,8-d_8 acid, (**14-3**) (0.60 g, 2.58 mmol) in toluene (4.5 mL) was added diphenylphosphoryl azide (0.81 g, 0.63 mL, 2.84 mmol) followed by triethylamine (0.40 mL, 2.84 mmol). The reaction mixture was heated to 90 °C for 2 hours. Benzyl alcohol (0.35 mL, 3.34 mmol) was added to the mixture which was heated at 90 °C overnight. The reaction mixture was allowed to cool to room temperature and was partitioned into EtOAc and aqueous saturated $NaHCO_3$ soln. The layers were separated and the organic phase was washed with aqueous saturated NH_4Cl soln, brine, dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was purified by silica gel chromatography (0-35-100% EtOAc/Hexanes – stain with CAMA). ^{1}H NMR shows desired product (**14-4**) along with benzyl alcohol impurity still present. Material was carried forward without further purification.

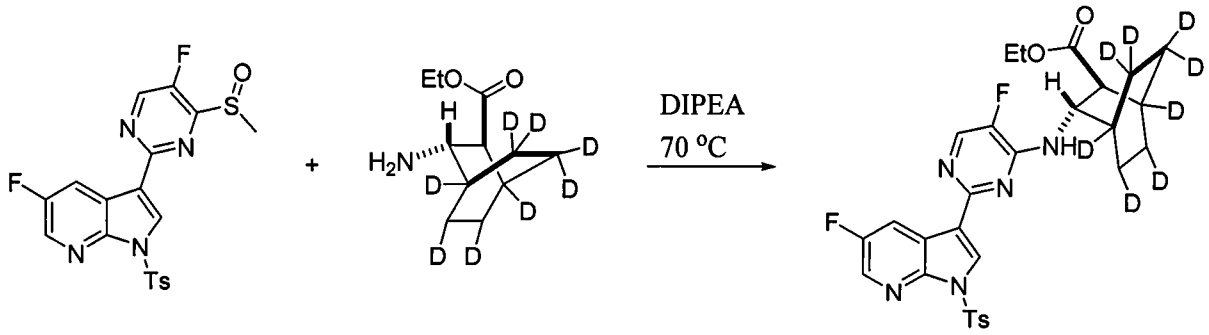
[0371] *14e: (+/-)-trans-ethyl-3-aminobicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (14-5)*



(+/-) 14-4 **(+/-) 14-5**

[0372] Palladium (0.052 g, 0.049 mmol) was charged into a hydrogenation vessel (under nitrogen atmosphere) and wet with approximately 5 mL of methanol. To the suspension was added a solution of (+/-)-*trans*-ethyl (2*S*, 3*S*)-3-(((benzyloxy)carbonyl)-amino)bicyclo[2.2.2]oct-5-ene-2-carboxylate-1,4,5,6,7,7,8,8-d_8 (**14-4**) (0.521 g, 1.547 mmol) in methanol (20 mL). The reaction mixture was subjected to hydrogenation (44 PSI) overnight. The pressure was vented and the catalyst was filtered off. All volatiles were removed *in vacuo*. The crude product (**14-5**) was used without further purification.

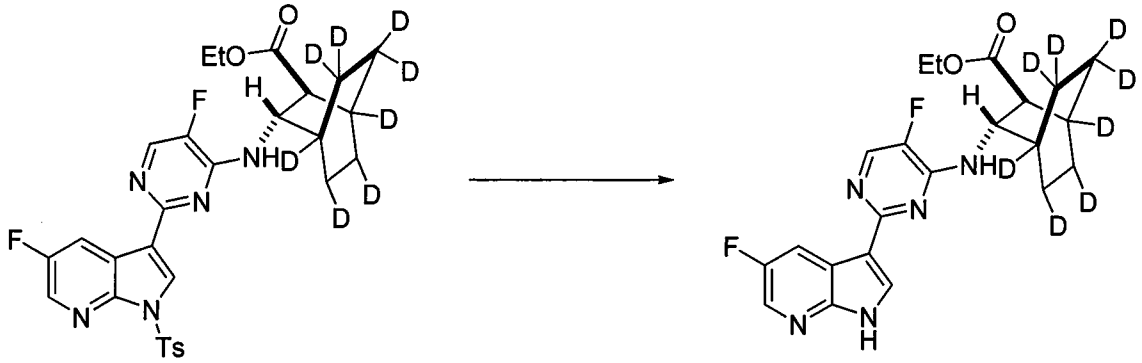
[0373] *14f: (+/-)-trans-ethyl-3-((5-fluoro-2-(5-fluoro-1-tosyl-1H-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)bicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (14-7)*



(+/-) 14-6 **(+/-) 14-5** **(+/-) 14-7**

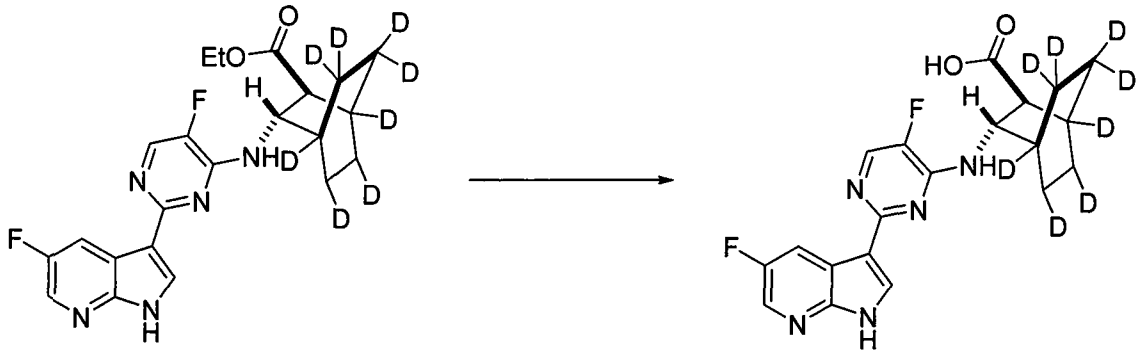
[0374] To a suspension of (+/-)-*trans*-ethyl (2*S*,3*S*)-3-aminobicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (**14-5**) (0.317 g, 1.547 mmol) and 5-fluoro-3-(5-fluoro-4-methylsulfinyl-pyrimidin-2-yl)-1-(*p*-tolylsulfonyl)pyrrolo[2,3-b]pyridine (**14-6**) (0.694 g, 1.547 mmol) in THF (10 mL) was added *N,N*-diisopropylethyl amine (0.808 mL, 4.641 mmol) and the reaction mixture was heated to 70 °C overnight. The reaction mixture was diluted with EtOAc and water. The layers were separated and the organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product (**14-7**) was purified by silica gel chromatography (0-100% EtOAc/Hexanes) to afford the desired product.

[0375] *14g: (+/-)-trans-ethyl-3-((5-fluoro-2-(5-fluoro-1H-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)bicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (**14-8**)*



[0376] To a solution of (+/-)-*trans*-ethyl (2*S*,3*S*)-3-((5-fluoro-2-(5-fluoro-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)bicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (**14-7**) (373 mg, 0.6325 mmol) in acetonitrile (6 mL) was added HCl (800 μL of 4 M solution in dioxane, 3.200 mmol). The reaction mixture was allowed to stir at room temperature for 2 hours. The reaction mixture was then heated to 80 °C for 6 hrs and then allowed to cool to room temperature and stirred overnight. LC/MS analysis shows reaction incomplete. An additional 6 ml of CH_3CN and 800 μl of 4N HCl/dioxane solution was added to the mixture. The reaction mixture was heated to 80 °C for 4 hours. All volatiles were removed at reduced pressure and the residue was diluted with EtOAc and aqueous saturated $NaHCO_3$. The layers were separated and the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc/Hexanes) to afford the desired product (**14-8**): ^{1}H NMR (300 MHz, *d*6-DMSO) δ 12.28 (s, 1H), 8.50 (dd, J = 9.8, 2.8 Hz, 1H), 8.23 (ddd, J = 12.6, 6.2, 2.7 Hz, 2H), 7.60 (d, J = 6.9 Hz, 1H), 4.73 (t, J = 6.5 Hz, 1H), 4.30 – 3.85 (m, 2H), 2.89 (d, J = 6.8 Hz, 1H), 1.59 – 0.96 (m, 4H).

[0378] *14h: (+/-)-trans-ethyl-3-((5-fluoro-2-(5-fluoro-1H-pyrrolo[2,3-b]pyridin-3-yl)pyrimidin-4-yl)amino)bicyclo[2.2.2]octane-2-carboxylic-1,4,5,5,6,6,7,8-d_8 acid* ***(1)***



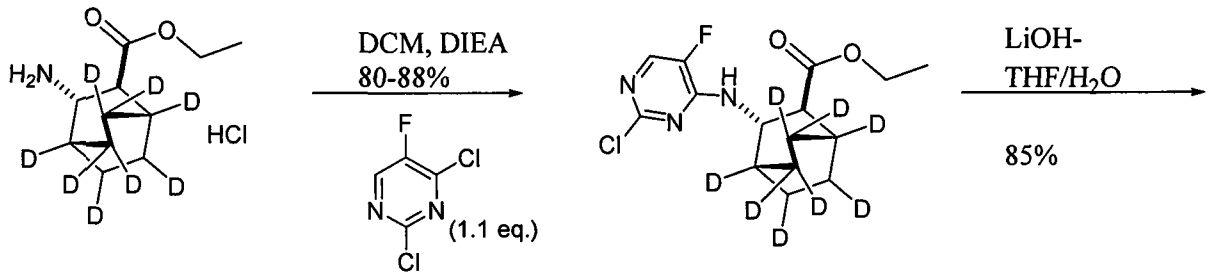
(+/-) 14-8 **(1)**

[0379] To a solution of (+/-)-*trans*-ethyl-3-((5-fluoro-2-(5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrimidin-4-yl)amino)bicyclo[2.2.2]octane-2-carboxylate-1,4,5,5,6,6,7,8-d_8 (**14-8**) (0.165 g, 0.379 mmol) dissolved in THF (3.0 mL) and methanol (1 mL) was added NaOH (1 mL of 2 M solution, 2.000 mmol) and the reaction mixture was stirred at room temperature for 3 hours. LC/MS analysis shows reaction is incomplete. The reaction mixture was warmed to 45 °C for 2 hours and then 55 °C for 30 minutes. The reaction mixture was diluted into aqueous saturated NH_4Cl solution. Several drops of 1N HCl were added to adjust the pH to approximately 6.5. The product was extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the desired product **(1)** (97.5% purity by NMR, LC/MS and HPLC): ^{1}H NMR (300 MHz, *d*6-DMSO) δ 12.30 (d, J = 14.2 Hz, 2H), 8.79 - 7.94 (m, 4H), 7.58 (s, 1H), 4.68 (s, 1H), 2.84 (s, 1H), 1.85 (d, J = 85.0 Hz, 1H), 1.58 - 1.05 (m, 2H).

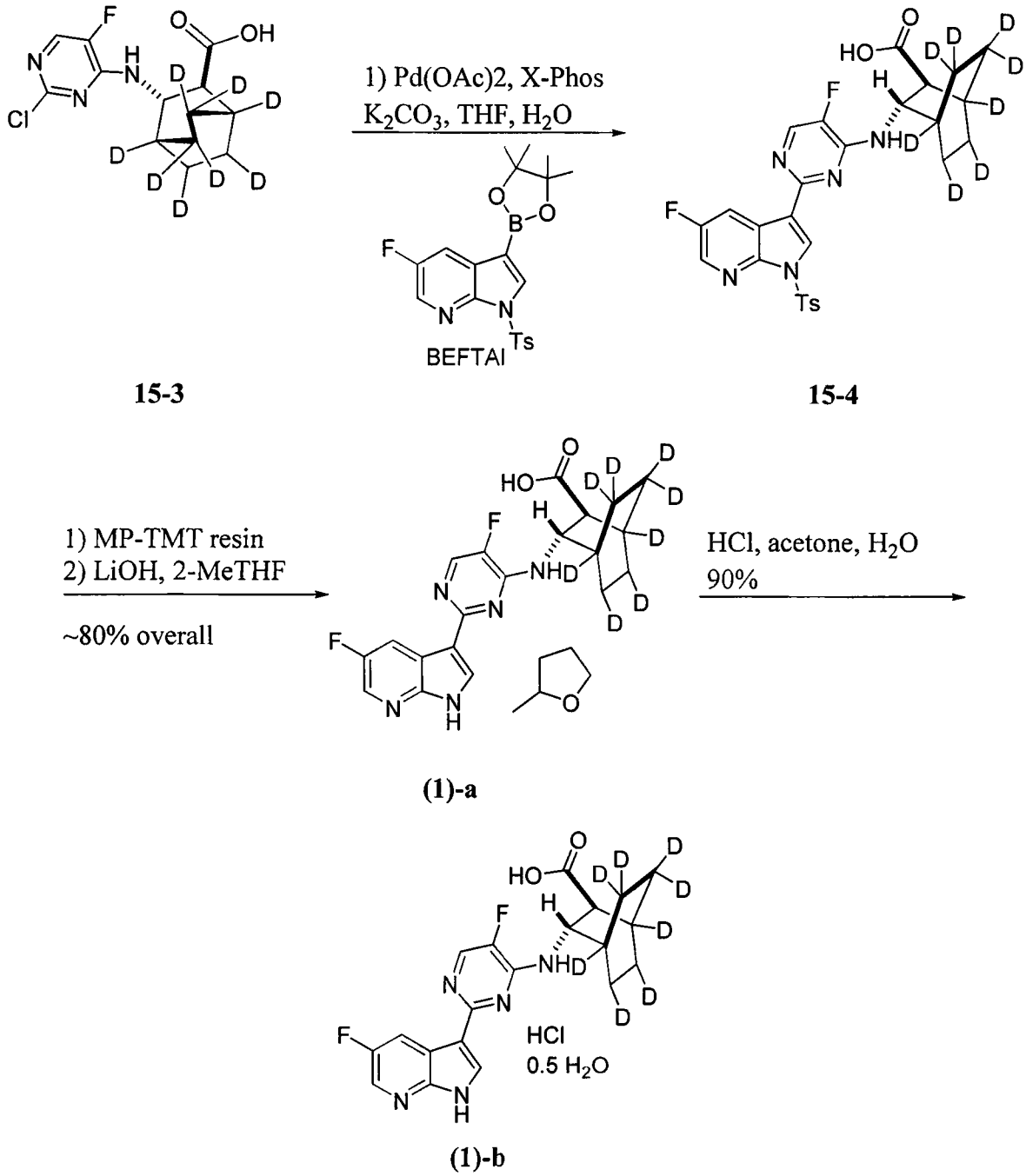
[0380] Example 15: Deuterium Enriched Compound (1).

[0381] Alternatively, deuterium enriched Compound **(1)** can be synthesized according to Scheme 2, below:

[0382] Scheme 2:



14-5 **15-2**

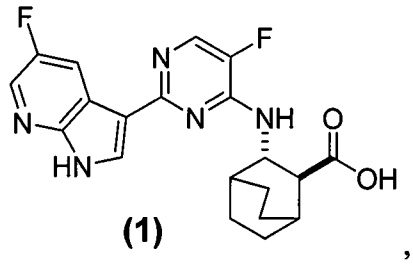


OTHER EMBODIMENTS

[0383] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof, wherein Compound (**1**) is represented by the following structural formula:



(1),

and wherein the polymorphic form is selected from the group consisting of:

Form A of HCl salt of Compound (**1**)•1/2H_2O,

Form F of HCl salt of Compound (**1**)•3H_2O,

Form D of HCl salt of Compound (**1**),

Form A of Compound (**1**), and

Form A of tosylate salt of Compound (**1**).

2. The polymorphic form of claim 1, wherein the polymorphic form is Form A of HCl salt of Compound (**1**)•1/2H_2O.

3. The polymorphic form of claim 2, wherein Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 10.5 ± 0.2, 5.2 ± 0.2, 7.4 ± 0.2, and 18.9 ± 0.2 in an X-ray powder diffraction pattern.

4. The polymorphic form of claim 3, wherein Form A of HCl salt of Compound (**1**)•1/2H_2O is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 25.2 ± 0.2, 16.5 ± 0.2, 18.1 ± 0.2, and 23.0 ± 0.2 in an X-ray powder diffraction pattern.

5. The polymorphic form of any of claims 2-4, wherein Form A of HCl salt of Compound (**1**)•1/2H_2O is characterized by one or more peaks corresponding to 29.2 ± 0.3 ppm, 107.0 ± 0.3 ppm, 114.0 ± 0.3 ppm, and 150.7 ± 0.3 ppm in a C^{13} SSNMR spectrum.

6. The polymorphic form of claim 5, wherein Form A of HCl salt of Compound (**1**)•1/2H_2O is further characterized by one or more peaks corresponding to 22.1 ± 0.3 ppm, 24.6 ± 0.3 ppm, 47.7 ± 0.3 ppm, and 54.8 ± 0.3 ppm in a C^{13} SSNMR spectrum.

7. The polymorphic form of claim 1, wherein the polymorphic form is Form F of HCl salt of Compound (**1**)•3H_2O.

8. The polymorphic form of claim 7, wherein Form F of HCl salt of Compound (**1**)•3H_2O is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 7.1 ± 0.2, 11.9 ± 0.2, 19.2 ± 0.2, and 12.4 ± 0.2 in an X-ray powder diffraction pattern.

9. The polymorphic form of claim 8, wherein Form F of HCl salt of Compound (**1**)•3H_2O is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 16.4 ± 0.2, 21.8 ± 0.2, and 23.9 ± 0.2 in an X-ray powder diffraction pattern.

10. The polymorphic form of any of claims 7-9, wherein Form F of HCl salt of Compound (**1**)•3H_2O is characterized by one or more peaks corresponding to 20.7 ± 0.3 ppm, 27.4 ± 0.3 ppm, 104.8 ± 0.3 ppm, 142.5 ± 0.3 ppm, and 178.6 ± 0.3 ppm in a C^{13} SSNMR spectrum.

11. The polymorphic form of claim 10, wherein Form F of HCl salt of Compound (**1**)•3H_2O is further characterized by one or more peaks corresponding to 154.3 ± 0.3 ppm, 20.3 ± 0.3 ppm, 132.3 ± 0.3 ppm, and 21.1 ± 0.3 ppm in a C^{13} SSNMR spectrum.

12. The polymorphic form of claim 1, wherein the polymorphic form is Form D of HCl salt of Compound (**1**).

13. The polymorphic form of claim 12, wherein Form D of HCl salt of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 5.8 ± 0.2, 19.5 ± 0.2, and 17.1 ± 0.2 in an X-ray powder diffraction pattern.

14. The polymorphic form of claim 13, wherein Form D of HCl salt of Compound (**1**) is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 5.3 ± 0.2, 10.5 ± 0.2, and 15.9 ± 0.2 in an X-ray powder diffraction pattern.

15. The polymorphic form of any of claims 12-14, wherein Form D of HCl salt of Compound (**1**) is characterized by one or more peaks corresponding to 29.4 ± 0.3 ppm, 53.4 ± 0.3 ppm, 113.3 ± 0.3 ppm, 135.4 ± 0.3 ppm, and 177.8 ± 0.3 ppm in a C^{13} SSNMR spectrum.

16. The polymorphic form of claim 15, wherein Form D of HCl salt of Compound (**1**) is further characterized by one or more peaks corresponding to 22.9 ± 0.3 ppm, 23.9 ± 0.3 ppm, 26.0 ± 0.3 ppm, and 31.6 ± 0.3 ppm in a C^{13} SSNMR spectrum.

17. The polymorphic form of claim 1, wherein the polymorphic form is Form A of Compound (**1**).

18. The polymorphic form of claim 17, wherein Form A of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 15.5 ± 0.2, 18.9 ± 0.2, and 22.0 ± 0.2 in an X-ray powder diffraction pattern.

19. The polymorphic form of claim 18, wherein Form A of Compound (**1**) is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 11.8 ± 0.2, 16.9 ± 0.2, 25.5 ± 0.2, and 9.1 ± 0.2 in an X-ray powder diffraction pattern.

20. The polymorphic form of any of claims 17-19, wherein Form A of Compound (**1**) is characterized by one or more peaks corresponding to 21.0 ± 0.3 ppm, 28.5 ± 0.3 ppm, 50.4 ± 0.3 ppm, 120.8 ± 0.3 ppm, 138.5 ± 0.3 ppm, and 176.2 ± 0.3 ppm in a C^{13} SSNMR spectrum.

21. The polymorphic form of claim 20, wherein Form A of Compound (**1**) is further characterized by one or more peaks corresponding to 30.1 ± 0.3 ppm, 25.9 ± 0.3 ppm, 22.8 ± 0.3 ppm, and 25.0 ± 0.3 ppm in a C^{13} SSNMR spectrum.

22. The polymorphic form of claim 1, wherein the polymorphic form is Form A of tosylate salt of Compound (**1**).

23. The polymorphic form of claim 22, wherein Form A of tosylate salt of Compound (**1**) is characterized by one or more peaks corresponding to 2-theta values measured in degrees of 7.2 ± 0.2, 9.3 ± 0.2, 13.7 ± 0.2, 14.3 ± 0.2, 14.7 ± 0.2, 16.9 ± 0.2, 18.7 ± 0.2 , 26.3 ± 0.2, and 26.9 ± 0.2 in an X-ray powder diffraction pattern.

24. The polymorphic form of claim 23, wherein Form A of tosylate salt of Compound (**1**) is further characterized by one or more peaks corresponding to 2-theta values measured in degrees of 6.0 ± 0.2, 28.0 ± 0.2, and 27.5 ± 0.2 in an X-ray powder diffraction pattern.

25. A pharmaceutical composition comprising a polymorphic form according to claim 1 and at least one pharmaceutically acceptable carrier or excipient.

26. The pharmaceutical composition of claim 25, wherein the polymorphic form is Form A of HCl salt of Compound (**1**)•1/2H_2O.

27. The pharmaceutical composition of claim 25, wherein the polymorphic form is Form F of HCl salt of Compound (**1**)•3H_2O.

28. The pharmaceutical composition of claim 25, wherein the polymorphic form is Form D of HCl salt of Compound (**1**).

29. The pharmaceutical composition of claim 25, wherein the polymorphic form is Form A of Compound (**1**).

30. The pharmaceutical composition of claim 25, wherein the polymorphic form is Form A of tosylate salt of Compound (**1**).

31. A method of reducing the amount of influenza viruses in a biological *in vitro* sample or in a subject, comprising administering to the sample an effective amount of a polymorphic form of Compound (**1**) according to any one of claims 1-24.

32. A method of inhibiting the replication of influenza viruses in a biological *in vitro* sample or in a subject, comprising administering to the sample an effective amount of a polymorphic form of Compound (**1**) according to any one of claims 1-24.

33. A method of treating influenza in a subject, comprising administering to the subject a therapeutically effective amount of a polymorph form of Compound (**1**) according to any one of claims 1-24.

34. The method of any one of claims 31-33, further comprising co-administering one or more additional therapeutic agents to the subject.

35. The method of claim 34, wherein the additional therapeutic agents include an anti-virus drug.

36. The method of claim 35, wherein the anti-virus drug is a neuraminidase inhibitor.

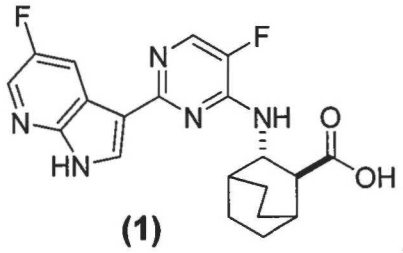
37. The method of claim 36, wherein the neuraminidase inhibitor is oseltamivir or zanamivir.

38. The method of claim 35, wherein the anti-virus drug is a polymerase inhibitor.

39. The method of claim 38, wherein the polymerase inhibitor is flavipiravir.

40. The method of any one of claims 31-39, wherein the influenza viruses are influenza A viruses.

41. A method of preparing Form A of HCl salt of Compound (**1**)•1/2H_2O, wherein Compound (**1**) is represented by the following structural formula:



(1), comprising:

mixing HCl with Compound (**1**) in a solvent system comprising water and one or more organic solvents, wherein the solvent system has a water activity of 0.05-0.85.

42. The method of claim 41, wherein the solvent system comprises one or more organic solvents selected from chlorobenzene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,2-dimethoxyethane, N,N-dimentylacetamide, N,N-dimethylformamide, 1,4-dioxane, 2-ethoxyethanol, formamide, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidone, nitromethane, pyridine, sulfolane, tetrahydrofuran (THF), tetralin, tolune, 1,1,2-trichloroethene and xylene, acetic acid, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, methylisobutyl ketone, 2-methyl-1-propanol, ethyl acetate, ethyl ether, ethyl formate, pentane, 1-pentanol, 1-propanol, 2-propanol, propyl acetate, or any combination thereof.

43. The method of claim 42, wherein the solvent system comprises one or more organic solvents selected from chlorobenzene, cyclohexane, 1,2-dichloroethane, dichloromethane, 1,2-dimethoxyethane, formamide, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, nitromethane, tetralin, xylene, toluene, 1,1,2-trichloroethane, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, ethanol, ethyl acetate, ethyl ether, ethyl formate, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, 2-methy-1-propanol, pentane, 1-propanol, 1-pentanol, 2-propanol, propyl acetate, tetrahydrofuran, methyl tetrahydrofuran, or any combination thereof.

44. The method of claim 42, wherein solvent system comprises one or more organic solvents selected from 2-ethoxyethanol, ethyleneglycol, methanol, 2-methoxyethanol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, ethanol, 1-pentanol, 1-propanol, 2-propanol, methylbutyl ketone, acetone, methylethyl ketone, methylisobutyl ketone, butyl acetate, isobutyl acetate, isopropyl acetate, methyl acetate, ethyl acetate, propyl acetate, pyridine, toluene, xylene, or any combination thereof.

45. The method of claim 42, wherein solvent system comprises one or more organic solvents selected from acetone, n-propanol, isopropanol, iso-butylacetate, acetic acid, or any combination thereof.

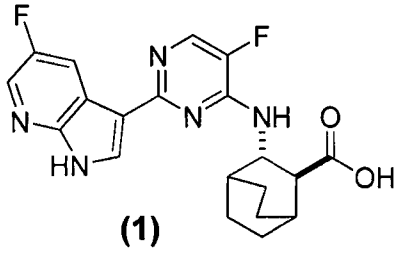
46. The method of claim 42, wherein the solvent system comprises one or more organic solvents selected from acetone or isopropanol.

47. The method of any one of claims 41-46, wherein the solvent system has a water activity value of from 0.4 to 0.6.

48. The method of any one of claims 41-47, wherein the mixing is performed at a temperature in a range from 5 °C to 75 °C.

49. The method of any one of clams 41-48, wherein the HCl is introduced as an aqueous solution having 30 wt% to 40 wt% HCl by weight of the aqueous solution.

50. A method of preparing Form F of HCl salt of Compound (**1**)•$3H_2O$, wherein Compound **1** is represented by the following structural formula:



(1), comprising:

(a) mixing HCl and Compound (**1**) in a solvent system comprising water, wherein the solvent system has a water activity of equal to, or greater than, 0.9; or

(b) stirring Form A of HCl salt of Compound (**1**)•$1/2H_2O$ in a solvent system that comprises water, wherein the solvent system has a water activity of equal to, or greater than, 0.9.

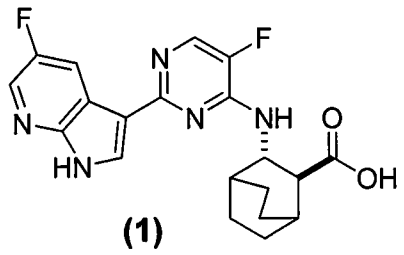
51. The method of claim 50, wherein the solvent system further comprises one or more organic solvents selected from chlorobenzene, cyclohexane, 1,2-dichloroethene, dichloromethane, 1,2-dimethoxyethane, N,N-dimentylacetamide, N,N-dimethylformamide, 1,4-dioxane, 2-ethoxyethanol, formamide, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, N-methylpyrrolidone, nitromethane, pyridine, sulfolane, tetrahydrofuran (THF), tetralin, tolune, 1,1,2-trichloroethene and xylene, acetic acid, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, methylisobutyl ketone, 2-methyl-1-propanol, ethyl acetate, ethyl ether, ethyl formate, pentane, 1-pentanol, 1-propanol, 2-propanol, propyl acetate, or any combination thereof.

52. The method of claim 51, wherein the solvent system further comprises one or more organic solvents selected from chlorobenzene, cyclohexane, 1,2-dichloroethane, dichloromethane, 1,2-dimethoxyethane, formamide, hexane, 2-methoxyethanol, methylbutyl ketone, methylcyclohexane, nitromethane, tetralin, xylene, toluene, 1,1,2-trichloroethane, acetone, anisole, 1-butanol, 2-butanol, butyl acetate, tert-butylmethyl ether, cumene, ethanol, ethyl acetate, ethyl ether, ethyl formate, heptane, isobutyl acetate, isopropyl acetate, methyl acetate, 3-methyl-1-butanol, methylethyl ketone, 2-methy-1-propanol, pentane, 1-propanol, 1-pentanol, 2-propanol, propyl acetate, tetrahydrofuran, or methyl tetrahydrofuran.

53. The method of claim 51, wherein the solvent system further comprises one or more organic solvents selected from 2-ethoxyethanol, ethyleneglycol, methanol, 2-methoxyethanol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, ethanol, 1-pentanol, 1-propanol, 2-propanol, methylbutyl ketone, acetone, methylethyl ketone, methylisobutyl ketone, butyl acetate, isobutyl acetate, isopropyl acetate, methyl acetate, ethyl acetate, propyl acetate, pyridine, toluene, xylene, or any combination thereof.

54. The method of claim 51, wherein the solvent system further comprises one or more organic solvents selected from *iso*-propanol, acetone or any combination thereof.

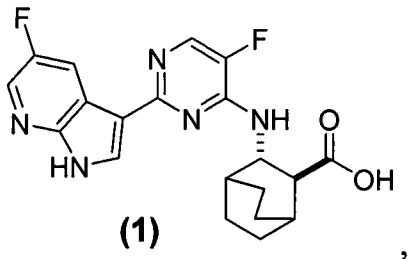
55. A method of preparing Form D of HCl salt of Compound (**1**), wherein Compound (**1**) is represented by the following structural formula:



(1) , comprising:

dehydrating Form A of HCl salt of Compound (**1**)•1/2H_2O.

56. A method of preparing Form A of Compound (**1**), wherein Compound (**1**) is represented by the following structural formula:



(1) , comprising:

(a) stirring an amorphous Compound (**1**) or a solvate of Compound (**1**) in a solvent system comprising water and ethanol.

57. The method of claim 56, wherein the stirring step is performed at a temperature in a range of 18 °C to 90 °C.

58. The method of either of claims 56 or 57, wherein the solvent system comprises 5 wt% to 15 wt% of water by weight of the solvent system.

59. The method of any one of claims 56-58, further comprising:

(b) stirring amorphous form of Compound (**1**) in nitromethane to form crystalline seed of Form A of Compound (**1**); and

(c) adding the crystalline seed of Form A of Compound (**1**) to the resulting mixture of the mixing step (a).

60. The method of any one of claims 56-59, wherein the stirring step (a) is performed at a refluxing temperature of the solvent system.

61. The method of any of claims 56-58, further comprising:

(b) stirring the amorphous form of Compound (**1**) in nitromethane to form crystalline seed of Form A of Compound (**1**); and

(c) cooling the resulting mixture of the mixing step (a) to a temperature in a range of 18 °C to 60 °C; and

(d) adding the crystalline seed of Form A of Compound (**1**) to the resulting mixture step (c).

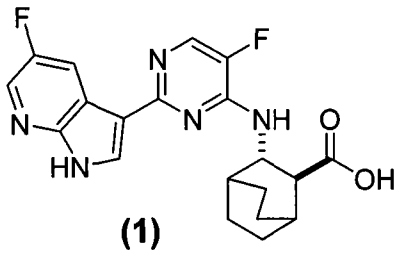
62. The method of claim 61, further comprising adding water, prior to the addition of crystalline seed of Form A of Compound (**1**), to the resulting mixture that has gone through

the refluxing step in an amount sufficient to impart the resulting solvent system with 15 wt% to 25 wt% of water after the addition of water.

63. The method of claim 61, further comprising adding water to the mixture that includes crystalline seed of Form A of Compound (**1**) in an amount sufficient to impart the resulting solvent system with 35 wt% to 45 wt% of water after the addition of water.

64. The method of claim 61, further comprising cooling the mixture that includes crystalline seed of Form A of Compound (**1**), after the addition of water, to a temperature of 0 °C to 10 °C.

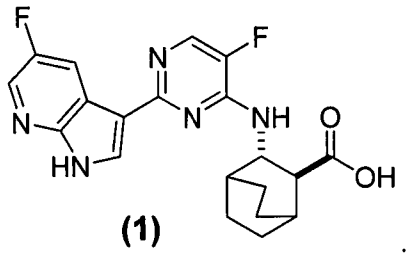
65. A method of preparing Form A of tosylate salt of Compound (**1**), wherein Compound (**1**) is represented by the following structural formula:



(1), comprising:

stirring a mixture of an amorphous Compound (**1**) or a solvate of Compound (**1**), *p*-toluenesulfonic acid, and a solvent system comprising acetonitrile.

66. A 2-methyl tetrahyrdrofuran solvate of Compound (**1**), wherein Compound (**1**) is represented by the following structural formula:



(1).

67. A dosage regimen comprising administering to a subject a polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof according to any one of claims 1-24 in a dosage amount of 100 mg to 1,600 mg, wherein the dosage amount is administered once, twice or three times per day.

68. The dosage regimen of claim 67, wherein the dosage amount is 300 mg to 1,600 mg.

69. The dosage regimen of claim 68, wherein the dosage amount is 600 mg to 1,200 mg.

70. The dosage regimen of claim 69, wherein the dosage is administered once per day.

71. The dosage regimen of claim 70, wherein the dosage amount is 600 mg or 800 mg.

72. The dosage regimen of claim 68, wherein the dosage amount is 300 mg to 900 mg.

73. The dosage regimen of claim 72, wherein the dosage is administered twice per day.

74. The dosage regimen of claim 68, wherein the dosage amount is 400 mg or 600 mg.

75. The dosage regimen of any one of claims 67-74, wherein the polymorphic form of Compound (**1**) or a pharmaceutically acceptable salt thereof is administered for duration of treatment of 1 day to an entire flu season.

76. The dosage regimen of claim 75, wherein the treatment duration is 3 days to 14 days.

77. The dosage regimen of claim 76, wherein the treatment duration is 3 days, 4 days, or 5 days.

78. The dosage regimen of any one of claims 67-77, wherein a loading dosage amount of 600 mg to 1,600 mg is administered to the subject on day 1 and a dosage amount of 400 mg to 1,200 mg is administered to the subject for the rest of the treatment duration.

79. The dosage regimen of claim 78, wherein a loading dosage amount of 900 mg to 1,600 mg is administered to the subject on day 1 and a dosage amount of 400 mg to 1,200 mg is administered to the subject for the rest of the treatment duration.

80. The dosage regimen of claim 79, wherein a loading dosage amount of 900 mg or 1,200 mg is administered to the subject on day 1 and a dosage amount of 600 mg to 800 mg is administered to the subject for the rest of the treatment duration.

81. The dosage regimen of claim 80, wherein a loading dosage amount of 900 mg is administered to the subject on day 1 and a dosage amount of 600 mg is administered once a day to the subject for the rest of the treatment duration.

82. The dosage regimen of claim 80, wherein a loading dosage amount of 1,200 mg is administered to the subject on day 1 and a dosage amount of 600 mg is administered once a day to the subject for the rest of the treatment duration.