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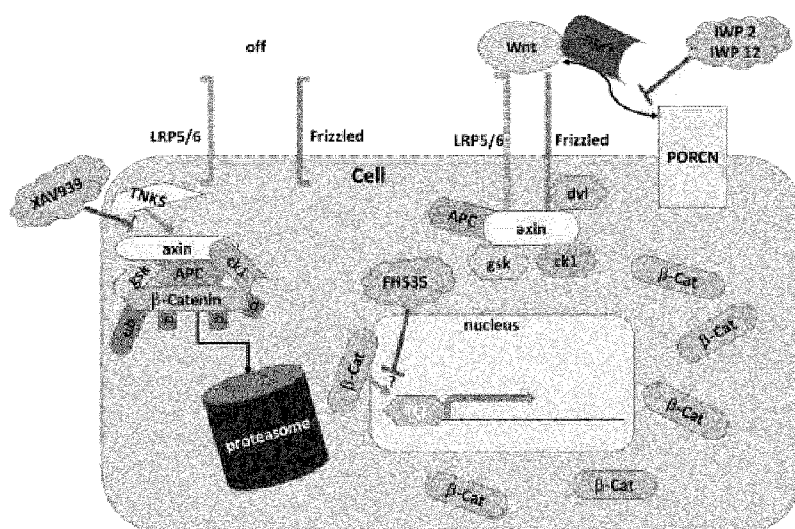


FIG. 1

(57) Abstract: The present disclosure relates to the use of a bioactive compound as an inhibitor of the Wnt/β-Catenin signalling pathway for inhibiting replication of Influenza A virus. The present disclosure also relates to a medicament for inhibiting replication of the Influenza A virus, the medicament comprising: bioactive compound that inhibits the Wnt/β-Catenin signalling pathway and one or more pharmaceutically acceptable excipients. The present disclosure also relates to a method of treating an Influenza A viral infection comprising, administering a bioactive compound that inhibits the Wnt/β-Catenin signalling pathway with one or more pharmaceutically acceptable excipients to a patient that has been diagnosed with an Influenza A viral infection.

COMPOSITIONS AND METHODS FOR TREATMENT OF INFLUENZA

TECHNICAL FIELD

The present invention relates to the field of treating viral infections. In particular,
5 the present invention relates to bioactive compounds that inhibit replication of seasonal and pathogenic avian strains of the Influenza A virus by inhibiting one or more parts of the Wnt/ β -Catenin signalling pathway.

BACKGROUND

Influenza A virus (IAV) is one of the most formidable human pathogens.
10 Worldwide, the annual death toll from IAV infections is close to 0.5 million. The United States of America annually sees roughly 140,000 hospitalisations with around 20,000 deaths from IAV infection. A broad host range and remarkable genetic plasticity mean that the typical means of effective treatment is vaccination. However, the current strategy for vaccinations is largely based on predicting what subtypes will be circulating in the
15 coming flu season. Unfortunately, the success rate of the vaccine can be very low. At least 18 hemagglutinin and 11 neuraminidase genes have been identified allowing a large number of combinations of these genes to produce new subtypes. Current antiviral drugs used to treat IAV are the neuraminidase inhibitors, oseltamivir and zanamivir, and the adamantane class of drugs, amantadine and rimantidine. These conventional drugs can
20 be effective. However, due to the emergence of resistance mutants, particularly among pathogenic avian strains, a rational design of new classes of antiviral compounds remains an ongoing effort. Furthermore, with the ease with which viral reassortment can lead to pathogenic human strains and there being a broad host range means that another IAV pandemic remains a likely threat. If antiviral compounds continue to target viral proteins,
25 then new strategies will always need to be developed to keep up with the evolution of the virus and the emergence of new subtypes.

Antiviral drugs that target host genes and pathways that are vital for virus replication may be more effective in combating IAV infections in the long term. Gene

silencing and interaction proteomics provide platforms for discovery of cellular pathways that can be targeted.

Identifying potential host factors for targeted inhibition has relied heavily on large data sets that are generated by gene silencing studies and quantitative proteomics.

- 5 Several publications have already presented attractive targets for inhibition and some of these targets have been characterized. However, there is still the need for continued efforts to find better therapeutics, be they vaccines or small molecule inhibitors..

- Complementary to targeting individual host genes, is the specific targeting of host pathways. A number of intracellular pathways vital for IAV replication have been
10 identified and are increasingly becoming targets for antiviral therapy. Some of these pathways include the Raf/MEK/ERK signal cascades. An example of one inhibitor with potential is the multi-kinase inhibitor ON108110, which demonstrated not only reduced IAV replication but inhibited replication of vesicular stomatitis virus (VSV) and Newcastle disease virus (NDV) with the reduction of IAV replication being greater than
15 90 %.

SUMMARY

Embodiments of the present disclosure relate to bioactive compounds that may provide anti-viral properties by selective targeting and / or regulation of the Wnt/ β -Catenin signalling pathway.

- 20 One of the known aspects of the Wnt/ β -Catenin signalling pathways regulates cytosolic levels of the β -Catenin protein. β -Catenin is a coactivator of a number of genes when it forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcriptional activators in the nucleus. β -Catenin acts as a downstream mediator of Wnt signaling.

- 25 An important feature of the Wnt signalling pathway is the controlled degradation of cytosolic β -Catenin by a destruction complex. The destruction complex comprises axin, adenomatosis polyposis coli (APC), glycogen synthase kinase 3-beta (GSK3 β) and casein kinase 1 α (CK1 α) In the absence of a Wnt signal, the destruction complex

assembles, the cytosolic β -Catenin is then constitutively phosphorylated and targeted to the proteasome. When the Wnt signal is switched on, the destruction complex dissociates and cytosolic β -Catenin will accumulate. Accumulated cytosolic β -Catenin may translocate to the nucleus, bind to TCF/LEF transcription factors and activate the expression of a number of Wnt responsive genes including ones that act as negative regulators of the pathway.

FIG. 1 provides a schematic representation of aspects of the Wnt/ β -Catenin signaling pathway by depicting the assembled destruction complex which targets cytosolic β -Catenin for degradation via the proteasome. A Wnt signal triggers the dissociation of the destruction complex which allows cytosolic β -Catenin to accumulate and translocate to the nucleus. As the downstream effector molecule of Wnt, nuclear β -Catenin complexes with TCF/LEF and together co-transcriptionally activate Wnt responsive genes.

Inappropriate activation of the Wnt pathway has been identified in multiple types of cancers including, but not limited to: colo-rectal carcinoma, breast carcinoma and hepatocellular carcinoma. As aberrations in this pathway are common to a variety of cancers, key stages in the pathway have been investigated as candidate targets for small molecule inhibition.

Several commercially available compounds have been developed as small molecule inhibitors and their cellular targets have been identified. FIG. 1 also shows the cellular targets of each of various bioactive compounds. To initiate a signal, PORCN adds a palmitoyl group to Wnt. Compounds IWP2 and IWP12 block this modification. The rate-limiting factor in the stability of the destruction complex is axin which is stabilised by Tankyrase. The bioactive compound XAV939 targets Tankyrase. Nuclear β -Catenin complexes with TCF and the bioactive compound FH535 targets TCF. Table 1 below provides some example small molecule inhibitor compounds that are bioactive and their cellular targets.

Table 1. Small molecule inhibitor compounds and their cellular targets.

Compound name	Cellular target
FH535	TCF/LEF
XAV939	Tankyrase 1
IWP2	Porcupine (PORCN)
IWR1	Tankyrase
IWP12	Porcupine (PORCN)

Embodiments of the present disclosure relate to the use of a bioactive compound that inhibits or downregulates one or more aspects of the Wnt/ β -Catenin signalling pathway for inhibiting replication of IAV.

- 5 Embodiments of the present disclosure relate to a medicament for inhibiting replication of IAV, the medicament comprising a bioactive compound that inhibits or downregulates one or more aspects of the the Wnt/ β -Catenin signalling pathway and one or more pharmaceutically acceptable excipients.

- 10 Embodiments of the present disclosure relate to a method of treating an IAV infection. The method comprises a step of administering a bioactive compound that inhibits or downregulates one or more aspects of the Wnt/ β -Catenin signalling pathway with one or more pharmaceutically acceptable excipients to a patient that has been diagnosed with IAV infection.

- 15 Embodiments of the present disclosure relate to a bioactive compound that inhibits or downregulates one or more aspects of the Wnt/ β -Catenin signalling pathway for use as an inhibitor, either prophylactic or responsive, of seasonal and pathogenic avian IAV strains.

The present disclosure may provide a connection between inhibition of the Wnt/ β -Catenin signalling pathway and IAV replication. It was observed that in addition

to seasonal IAV inhibition, the bioactive compound FH535 inhibited avian strain H5N1 by 90 % in A549 cells and both H5N1 and H7N9 strains by 99 % and 90 % respectively in human bronchial epithelial cells (HBEC). It was also observed that the bioactive compound FH535 acts early on and with a biological effect that is prolonged throughout the Wnt/ β -Catenin signalling pathway.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings:

FIG. 1 is a schematic representation of an example of the Wnt/ β -Catenin signaling pathway;

10 **FIG. 2** shows examples of experimental data that relate to embodiments of present disclosure;

FIG. 2A shows an example of cytopathic effect of the IAV strain PR8 on examples of human bronchial epithelial cells (HBEC); **FIG. 2B** shows an example of immunoblot-assay time course results of influenza NS-1 protein production in example A549 cells and example HBEC cells that were infected with IAV strain PR8 at a multiplicity of infection (MOI) of 5; and **FIG. 2C** shows a growth curve of the IAV strain PR9 in HBEC cells infected at a MOI of 0.01, the plaque assay results were performed in dog kidney epithelial cells (MDCK);

20 **FIG. 3** shows an example of A549 cell and HBEC cell viability results as determined by the WST-1 viability assay, in the presence of various bioactive compounds: **FIG. 3A** shows cell viability during IWP12 treatment; **FIG. 3B** shows cell viability during IWP2 treatment; **FIG. 3C** shows cell viability during XAV939 treatment; and **FIG. 3D** shows cell viability during FH535 treatment;

25 **FIG. 4** shows examples of titer of influenza strain PR8 data from infected A549 cells and HBEC cells that were treated with bioactive compounds: **FIG. 4A** shows data from cells treated with IWP12; **FIG. 4B** shows data from cells treated with IWP2; **FIG. 4C**

shows data from cells treated with XAV939; and **FIG. 4D** shows data from cells treated with FH535;

FIG. 5 shows examples of viral inhibition data from cells infected with seasonal IAV strains and treated with the bioactive compound FH535: **FIG. 5A** shows inhibition of
5 Influenza A viral strains in cells treated with FH535 **FIG. 5B** shows inhibition of avian viral strains in cells treated with FH535;

FIG. 6 shows examples of expression and localization data of proteins from cells that were infected with virus: **FIG. 6A** shows examples of expression and localization results of the Influenza NP protein in cells; **FIG. 6B** shows examples of co-localization results
10 of the influenza NP protein and the CRM1 protein; and

FIG. 7 shows examples of quantification results of the Influenza NP protein in A549 cells infected with IAV strain PR8 compared with PR8 infected A549 cells that were treated with the bioactive compound FH535: **FIG. 7A** shows an example of NP and GAPDH protein levels measured in control (C) and FH 535 treated (T) cells via Western
15 blotting techniques; and **FIG. 7B** is a bar graph that shows the values of the NP expression in control and FH535 treated cells at 16 hours, 20 hours and 42 hours.

DETAILED DESCRIPTION

Embodiments of present disclosure relate to a use of a bioactive compound that regulates an aspect of the Wnt/ β -Catenin signaling pathway to inhibit viral replication.
20 The bioactive compounds may be small molecule inhibitors or downregulator of the Wnt/ β -Catenin signaling pathway.

In some embodiments of the present disclosure, the following compounds may be bioactive compounds that act as small molecule inhibitors of one or more aspects of the Wnt/ β -Catenin signaling pathway: FH535 with a cellular target of TCF/LEF;
25 XAV939 with a cellular target of Tankyrase 1; IWP2 with a cellular target of Porcupine (PORCN); IWR1 with a cellular target of Tankyrase; IWP12 with a cellular target of PORCN; IWP-2 with a cellular target of Porcupine; JW74 with cellular targets of Tankyrases 1, 2; JW55 with cellular targets of Tankyrases 1, 2; Okadaic acid with a

cellular target of PP2A phosphate; Tautomycin with a cellular target of PP1 phosphatase; SB2390963 with a cellular target of p38 MAPK; SB203580 with a cellular target of p38 MAPK; 2-[4-(4-fluorophenyl)piperazin-1-yl]-6-methylpyrimidin-4(3H)-one with a cellular target of Tankyrases 1,2; PJ34 with cellular targets of Tankyrases 1, 2; 5 Nicosamide with a cellular target of Dvl-2; Cambinol with a cellular target of SIRT1; Sulindac with a cellular target of the PDZ domain of Dishevelled; 3289-8625 with a cellular target of dishevelled; Scaffold A for a series of analogues with a cellular target of Dishevelled; Scaffold B for a series of analogues with a cellular target of Dishevelled; J01-017a with a cellular target of Dishevelled; NSC668036 with a cellular target of 10 Dishevelled; Filipin with a cellular target of caveolin-mediated endocytosis; IC261 with cellular targets of CK1 ϵ and δ ; PF670462 with cellular targets of CK1 ϵ and δ ; Bosutinib with a cellular target of Src kinase; PHA665752 with a cellular target of c-Met; Imatinib with various tyrosine kinase cellular targets; ICG-001 with a cellular target of CREB binding protein (CBP); Ethacrynic acid derivatives with a cellular target of Lef- 15 1; PKF115-584 with a cellular target of β -Catenin; PNUI-74654 with a cellular target of β -Catenin; PKF118-744 with a cellular target of β -Catenin; CGP049090 with a cellular target of β -Catenin; PKF118-310 with a cellular target of β -Catenin; ZTM000990 with a cellular target of β -Catenin; BC21 with a cellular target of β -Catenin; GDC-04 with a cellular target of PI3K; and Rp-8-Br-cAMP with a cellular target of PKA. The structures 20 of these compounds can be found in Voronkov and Krauss (2013) *Wnt/beta-catenin Signalling and Small Molecule Inhibitors* Current Pharmaceutical Design 19:634-664, the entire disclosure of which is incorporated herein by reference.

Some embodiments of the present disclosure relate to bioactive compounds that inhibit or downregulate the recruitment of β -Catenin to target genes of the TCF/LEF 25 family of transcription factors. Some embodiments of the present disclosure relate to bioactive compounds that inhibit or downregulate the TCF/LEF family or transcription factors or receptors therefor. By way of example, FH535 is a bioactive compound that inhibits or downregulates the receptors for the TCF/LEF family of transcription factors.

Embodiments of the present disclosure relate to a medicament for inhibiting 30 replication of IAV, the medicament comprising a bioactive compound that inhibits or

downregulates one or more aspects of the the Wnt/ β -Catenin signalling pathway and one or more pharmaceutically acceptable excipients.

Embodiments of the present disclosure relate to a method of treating an IAV infection. The method comprises a step of administering a bioactive compound that
5 inhibits or downregulates one or more aspects of the Wnt/ β -Catenin signalling pathway with one or more pharmaceutically acceptable excipients to a subject that has been diagnosed with IAV infection or a predisposition for IAV infection. In some
10 embodiments of the present disclosure, the method of treating the IAV infection may be a prophylactic administration in the absence of any IAV infection indications, to prevent the occurrence of and/or the spreading of an infection within a subject or between subjects, when at least one subject has a predisposition to developing an IAV infection. A subject may develop a predisposition to developing an IAV infection by exposure to a known source of IAV, for example a known source may be another subject that has been diagnosed with an IAV infection.

15 In other embodiments of the present disclosure, the method of treating the IAV infection may be reactive in a subject that demonstrates IAV infection indications. The reactive method of treatment is for preventing the severity of the IAV infection, reduce the time the infected subject is afflicted by the IAV infection and to reduce spreading of the IAV infection to other subjects.

20 The method of treating an IAV infection comprises administration of the bioactive compound by at least one of: oral administration; injection, including intravenous, intramuscular, sub-cutaneous or intrathecal injection routes; sublingual placement; buccal placement; rectal insertion; vaginal insertion; ocular placement; otic placement; topical application, including cutaneous application, nasal spray, oral spray,
25 oral inhalation, oral or nasal inhalation of a nebulized form of the bioactive compound; transdermal application or combinations thereof.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

As used herein, the term “about” refers to an approximately +/-10% variation
5 from a given value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

As used herein, the term “bioactive compound” refers generally to compounds and pharmaceutically acceptable salts and derivatives thereof that can inhibit or downregulate the biological activity of one or more aspects of the Wnt/ β -Catenin
10 signaling pathway.

As used herein, the term “excipient” herein means any substance, not itself a therapeutic agent, which may be used in a composition for delivery of at least one of the bioactive compounds described herein and the like to a subject to improve its handling or storage properties or to permit or facilitate formation of a dose unit of the composition
15 (e.g., formation of a topical hydrogel which may then be optionally incorporated into a transdermal patch). Excipients include, by way of illustration and not limitation, binders, disintegrants, taste enhancers, solvents, thickening or gelling agents (and any neutralizing agents, if necessary), penetration enhancers, solubilizing agents, wetting agents, antioxidants, lubricants, emollients, substances added to mask or counteract a disagreeable odor, fragrances or taste, substances added to improve appearance or texture
20 of the composition and substances used to form a pharmaceutical composition. Any such excipients can be used in any dosage forms according to the present disclosure. The foregoing classes of excipients are not meant to be exhaustive but merely illustrative as a person of ordinary skill in the art would recognize that additional types and combinations of excipients could be used to achieve the desired goals for delivery of at
25 least one of the bioactive compounds described herein and the like.

As used herein, the term “effective amount” refers to an amount effective, at dosages and for periods of time necessary to achieve the desired results (e.g. prophylaxis of microbial infections). Effective amounts of a molecule may vary according to factors

such as the disease state, age, sex, weight of the subject. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

5 As used herein, the term “IAV” refers to the Influenza A virus.

 As used herein, “inhibit”, “inhibiting”, and “inhibition” refers to a decrease in an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response, condition,
10 or disease as compared to the native or control level. Thus, the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%; 100%, or any amount of reduction in between the specifically recited percentages, as compared to native or control levels.

 As used herein, the terms “multiplicity of infection” and “MOI” refer to a ratio of a viral load to target cells.

15 As used herein, the term “nebulized form” refers to a misted form or alternatively, in an aerosol form.

 As used herein the phrase “one or more aspects of the Wnt/ β -Catenin signaling pathway” refers to any sequence of nucleic acids or amino acids or any other biomolecule that participates in the Wnt/ β -Catenin signaling pathway. The one or more aspects of the
20 Wnt/ β -Catenin signaling pathway can be cellular targets of one or more bioactive compounds of the present disclosure.

 As used herein, “pharmaceutical composition” includes any composition for administration of at least one of the bioactive compounds disclosed herein to a subject in need of treatment for exposure to one or more strains of the Influenza A virus.
25 Pharmaceutical compositions may include various excipients, carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to at least one of the bioactive compounds disclosed herein. Pharmaceutical compositions may also

additionally include one or more further active ingredients such as antimicrobial agents, anti-inflammatory agents, anaesthetics, analgesics, and the like.

As used herein, the term “prophylactic administration” refers to the administration of any composition to a subject, in the absence of any infection
5 indications, to prevent the occurrence of and/or the spreading of an infection within the subject’s body.

As used herein, the term “subject” means any target of administration. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human. The term does not denote a particular age or sex. Thus, adult, juvenile, and newborn
10 subjects, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term “patient” includes human and veterinary subjects.

As used herein, the term “therapeutically effective” refers to an amount of a treatment or composition that is used of sufficient quantity to ameliorate one or more
15 causes or symptoms of a disease or disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination. The “therapeutically effective amount” will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be treated.

As used herein, the terms “treatment”, “treating”, and the like, refer to obtaining
20 a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment”, as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from
25 occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

Embodiments of the present disclosure will now be described with reference to FIG. 1 through FIG. 7, which show representations of embodiments according to the present disclosure.

The present disclosure relates to use of a bioactive compounds that regulate the
5 Wnt/ β -Catenin signaling pathway and inhibit viral replication.

One embodiment relates to the use of a bioactive compound FH535 for inhibiting IAV replication. The bioactive compounds FH535 can also be referred to as 2,5-dichloro-N-(2-methyl-4-nitrophenyl)benzenesulfonamide with a formula of $C_{13}H_{10}Cl_2N_2O_4S$. FH535 may inhibits the Wnt signalling pathway by blocking
10 activation of the TCF transcription factor, which reduces replication of seasonal IAV strains and pathogenic avian IAV strains.

Another embodiment of the present disclosure relates to a medicament for inhibiting replication of IAV, the medicament comprises a bioactive compound that inhibits the Wnt/ β -Catenin signalling pathway and one or more pharmaceutically
15 acceptable excipients.

Another exemplary embodiment of the present disclosure relates to a method of treating an IAV infection. The method comprises a step of administering a bioactive compound that inhibits the Wnt/ β -Catenin signalling pathway with one or more pharmaceutically acceptable excipients to a patient that has been diagnosed with
20 Influenza A viral infection.

EXAMPLES

Example 1

For *in vitro* analysis, A549 cells and Madin-Darby canine kidney (MDCK) epithelial cells were maintained at 37° C (5 % CO₂) in 1 X Dulbecco's modified Eagle
25 medium (DMEM) supplemented with L-Glutamine, sodium pyruvate, non-essential amino acids, 10 % fetal bovine serum (FBS) (A549) and 5 % FBS (MDCK). Human bronchial epithelial cells HBEC-3KT (ATCC® catalogue # CRL-4051, "HBEC"; ATCC is a registered trademark of the American Type Culture Collection, Manassas, VI) were

maintained at 37° C (5 % CO₂) in Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030) supplemented with Bronchial Epithelial Growth kit (ATCC® PCS-300-040). The following stocks of influenza strains were assessed: A/Puerto Rico/8/34 (H1N1; PR8), A/New York/55/2004 (H3N2; NY55), A/New Caledonia/20/1999/ (H1N1; NC) 5 A/Indonesian/5/2005 (H5N1), A/Anhui/1/2013 (H7N9) and A/Brisbane/59/2007 (H1N1; B59). These stocks were grown in MDCK cells infected at a multiplicity of infection (MOI) of 0.01 for about 42 hours. Supernatants from all but H5N1 and H7N9 were collected, clarified at 1000 rpm for 10 minutes then concentrated at 60,000 X g for 120 minutes. Concentrated stocks were stored in 5 % glycerol at -80° C.

10 **Example 2**

Mouse monoclonal antibodies to the IAV proteins NP and NS1 were made based upon the protocol disclosed in Rahim, M. N. et al., (2013, *Generation and characterization of a new panel of broadly reactive anti-NS1 mAbs for detection of influenza A virus*. J Gen Virol, v. 94, n. Pt 3, p. 593-605, ISSN 0022-1317) the entire 15 disclosure of which is incorporated herein by reference. Rabbit polyclonal anti-CRM1 (H-300: sc-5595) was obtained from SANTA CRUZ BIOTECHNOLOGY® (SANTA CRUZ BIOTECHNOLOGY is a registered trademark of Santa Cruz Biotechnology Inc. of 2145 Delaware Avenue, Santa Cruz, CA, USA). Secondary horse radish peroxidase (HRP) conjugated donkey anti-rabbit (SA1-200) and goat anti-mouse (G21040) 20 antibodies were obtained from Thermo Fisher Scientific, Canada for Western Blots. Cy5 conjugated goat anti-rabbit (A10524) and Alexa fluor-488 goat anti-mouse (A28175) secondary antibodies for immunofluorescence microscopy were obtained from Thermo Fisher Scientific, Canada.

Example 3

The bioactive compounds used in this study were purchased from SIGMA-ALDRICH® Co. (SIGMA-ALDRICH is a registered trademark of SIGMA-ALDRICH BIOTECHNOLOGY L.P. of 3050 Spruce Street, St. Louis, MISSOURI 63103, USA) and purified to a high-performance liquid chromatography (HPLC) standard of > 98 %. The bioactive compounds were distributed in a powder form and dissolved in dimethyl sulfoxide (DMSO) to the following concentrations: XAV939 (16 mM), IWP12 (11.9 mM), IWP2 (10.71), IWR1 (12.2 mM) and FH535 (13.8 mM). Cell viability in the presence of all compounds was measured by the WST-1 protocol according to the instructions provided by the kit manufacturer Roche of Laval, Quebec, Canada.

Example 4

Seasonal strains NY55, NC, B59 and PR8 were grown in MDCK cells and concentrated to > 108 PFU/ml for experiments. Plaque titration assays were done on MDCK cells using avicel as the overlay according to the protocols disclosed in Matrosovich, M. et al. (2006, *New low-viscosity overlay medium for viral plaque assays*. Virol J, v. 3, p. 63, 2006. ISSN 1743-422x.).

Example 5

For virus inhibition studies with the bioactive compounds, cells were passaged to be > 90% confluent the following day; the compounds were added directly to the media of cultured cells. A549 and HBEC cells were pre-treated for 16 hr with various concentrations of each bioactive compound (IWP12, IWP2, IWR1, FH535). Following pre-treatment, media was removed and cells were washed with un-supplemented 1X DMEM and infected with one strain of the virus at various MOIs (0.001, 0.01, 0.1, 1 and 10) for different experiments. The compounds were returned to the infection media (1 X DMEM supplemented with L-Glutamine, sodium pyruvate and non-essential amino acids) post-adsorption and infected cells were incubated at 35 °C for 42 hours. Infectious supernatants were collected and stored at -80 °C in 5 % glycerol (v/v) for plaque titration. Infection of pre- and post-treated A549 and HBEC cells with pathogenic avian influenza viruses was performed under strict bio-containment conditions (BSL 3) such as those

found at the Zoonotics division, National Microbiology labs, Public Health Agency of Canada.

Example 6

For analysis by Western blot, supernatant from the virus inhibition assays was removed and infected cell monolayers were washed twice with 1X phosphate-buffered saline (PBS). Cells were harvested in PBS into 1.5 ml tubes and pelleted at 14,000 x g for 10 seconds. The PBS was removed and the pellets were incubated on ice in lysis buffer (0.5 % NP-40/complete protease inhibitor cocktail) for 15 minutes. Cell pellets were centrifuged at 14,000 x g for 7 minutes and the clarified supernatants were removed to a new 1.5 ml tube and stored at -200 °C. Total protein concentration was determined by a bicinchoninic acid (BCA) assay (BIO-RAD of Hercules, California, USA) as per the manufacturer's protocol. Equal concentrations of proteins were mixed with 4 X Laemli buffer containing dithiothreitol (DTT) and boiled for 5 minutes at 95 °C. Protein lysates were added to 15 % sulfate polyacrylamide (SDS) gels and resolved at 160 V for 55 minutes. Protein was then transferred to polyvinylidene fluoride (PVDF) membranes at a constant voltage of 90 V for 60 minutes in 1X Towbin's transfer buffer. PVDF membranes were blocked overnight in 5 % (w/v) milk/PBS with Tween-20 or in 4 % bovine serum albumin (BSA)/PBS and stained with primary and secondary antibodies for Influenza nucleoprotein (NP) and GAPDH. Densitometry assays of resolved bands were normalised to GAPDH.

Example 7

For immunofluorescence microscopy studies, A549 cells were cultured onto 22 mm X 22 mm glass cover slips in 6-well plates prior to overnight pre-treatment with 3 µM of compound FH535. Cells were infected with influenza strain PR8 at an MOI of 10, infection media including 3 µM of FH535 was added back to the cells and they were incubated at 35 °C (5% CO₂) until times of processing. Cover slips were washed with PBS then fixed with 2 % formaldehyde (v/v) for 10 minutes at room temperature. Fixing solution was removed with PBS washes and the cells were permeabilised (0.5 % Triton X-100, 2 % BSA, PBS) at room temperature for 15 minutes. After further washes with

PBS, cells were blocked with 4 % BSA (w/v) for 30 minutes at room temperature. Immediately following blocking, cells were incubated with primary antibody in 4 % BSA for 45 minutes (mouse monoclonal anti NP; rabbit polyclonal anti CRM1) at room temperature. After further washes in PBS, cells were incubated with Alexa flour-488
5 conjugated secondary antibody (nucleoprotein - NP) and CY3 conjugated secondary antibody (CRM1) for 45 minutes at room temperature. During the final PBS wash, nuclei were stained with DAPI for 5 minutes and cover slips were mounted onto microscope slides using Immu-Mount™ (Fisher Scientific of Pittsburgh, Pennsylvania, USA) and stored at 4 °C. Time points examined were 12, 20, 30 and 42 hours post infection.

10 **Example 8**

Most known *in vitro* studies of IAV replication are performed in either continuously cultured human lung A549 cells or in MDCK cells. It is known, however, that primary cells behave differently from continuously cultured transformed cells. The inventors observed that IAV induces a differential proteomic response in primary human
15 bronchial airway epithelial (HBAE) cells compared to A549 cells. Most primary cells, including HBAE, are slow growing and limited in their passage capacity. Thus, alternative primary-like cell models were sought to test the general applicability of the Wnt/ β -Catenin signaling pathway inhibitors to inhibit IAV.

ATCC® offers numerous cell types, including continuously cultured, primary
20 cells, and human telomerase-immortalized (hTERT) cells.

The inventors tested human bronchial epithelial cells (HBEC) and found they doubled approximately daily and could be maintained with no observable phenotypic differences up to passage number 20. The HBEC cells were tested and it was found that they supported IAV growth (as shown in FIG. 2). FIG. 2A shows the cytopathic effect
25 in HBEC cells in either mock (top row) or infected with PR8 (bottom row) over time. FIG. 2B shows the immunoblot evaluation of IAV NS-1 protein production in A549 and HBEC cells infected at MOI = 5 and assayed at indicated times. FIG. 2C depicts a growth curve of PR8 production in HBEC cells after MOI=0.01 infection; plaque assay performed in MDCK cells.

IAV-induced cytopathic effect manifested by 48 hours post-infection (hpi) (FIG. 2A), expression of the viral non-structural protein NS-1 was apparent by 6 hpi (FIG. 2B), which validates successful progeny viral protein production, and infectious progeny virions were produced (FIG. 2C). The inventors concluded that HBEC cells can support IAV replication. Thus, the inventors used both the hTERT and transformed A549 cells in further work.

Example 9

The viability of A549 cells 10 and HBEC cells 12 in the presence of the four bioactive compounds (IWP12, IWP2, XAV939, FH535) was determined by WST-1 assay. FIG. 3A-3D show A549 cell 10 and HBEC cell 12 viability in the presence of the bioactive compounds, as determined by the WST-1 assay. The assay was performed in 96 well plates and viability at each concentration was calculated as a percentage of the control (untreated cells). Error bars represent the standard error from the average of 5 technical replicates. Cells were cultured in the presence of the bioactive compounds for 48 hours prior to addition of WST-1 reagent. All of the bioactive compounds were well tolerated in both A549 cells 10 and in HBEC cells 12. The TD50 value for FH535 was calculated as 100 μ M in A549 cells 10 and 50 μ M in HBEC cells 12 (FIG. 3).

Example 10

For the IAV inhibition studies, A549 cells 10 and HBEC cells 12 were treated with increasing concentrations of the bioactive compounds that target different stages in the Wnt signalling pathway. Virus inhibition by the bioactive compounds was determined by plaque titration on MDCK cells. FIG. 4A through FIG. 4D show the titres of influenza strain PR8 from infected A549 cells 10 and HBEC cells 12 treated with bioactive compounds at the various micro-molar concentrations shown. Cells were treated with each bioactive compound for 16 hours and infected with virus for 39 – 42 hours. Supernatants were harvested and titrated onto MDCK cells to determine the virus titre. Error bars represent the standard error determined from the averages of three independent experiments. Initial inhibition studies were carried out using PR8 to determine which compound and what dose demonstrated inhibition of viral replication.

Compounds XAV939, IWP2 and IWP12 demonstrated no observed effect on viral replication even at the highest concentrations tested (FIG. 4). Compound FH535 reduced virus titre by 3 log₁₀ in both cell lines between 3 µM and 5 µM and below the limit of detection above 5 µM. The FH535 viral inhibitory ED₅₀ was calculated as 0.01 µM in A549 cells and as 0.2 µM in HBEC, giving therapeutic indices of about 10,000 in A549 cells 10 and 250 in HBEC cells 12. Thus, a concentration of 3 µM FH535, which inhibited all tested strains > 90% in, was selected for further use in the following examples.

Example 11

FH535 effects upon additional seasonal IAV strains were tested in A549 cells 10, HBEC cells 12 and MDCK cells 14. FIG. 5 shows FH535 tested on seasonal influenza strains at 3 µM concentration. Inhibition of PR8 was used as a positive control. Similar reduction in titres can be observed with the other influenza strains in both cell types as is seen with PR8. Inhibition was also tested in MDCK indicator cells 14 (FIG. 5A). Error bars are determined as the standard error from 3 independent experiments. FIG. 5B shows FH535 inhibits pathogenic avian strains of influenza and potency of inhibition is cell type dependent.

HBEC cells 12 and A549 cells 10 were infected with H5N1 and H7N9 viruses alongside PR8 and New Caledonia strains (positive controls) at an MOI of 0.001. Supernatants were harvested 42 hours post-infection and titrated on MDCK cells 14 to determine the tissue culture infectious dose (TCID₅₀) titres. Inhibition, determined by plaque assays after treatment, ranged from ~ 95 – 99.9% for each of the tested H1N1 and H3N2 IAV strains in all cell types. Inhibition of pathogenic avian viruses by FH535 was assayed in both A549 cells 10 and HBEC cells by TCID₅₀ assay in MDCK cells 14 (FIG. 5B). The degree of inhibition of the pathogenic avian strains of influenza was cell specific. There was a significant reduction in the titre of H5N1 in A549 cells 10 but not to the same extent as both H5N1 and H7N9 in HBEC cells 12 (FIG. 5B).

Example 12

A549 cells were treated with the bioactive compound FH535 at 3 μ M and infected with PR8 at an MOI of 10 to observe protein localization. FIG. 6A and FIG. 6B show the expression and localisation of the IAV protein NP. A549 cells were seeded onto 22 mm glass cover slips and pre-treated for 16 hours with 3 μ M of FH535 and post-treated with the same concentration post-infection with PR8 at an M.O.I of 10. At the time points shown, the cells were stained with anti-NP (FIG. 6A and FIG. 6B) and anti-CRM1 (FIG. 6B) primary and the appropriate secondary antibodies.

FIG. 6A (20X magnification) shows that infection of FH535-treated cells was not as efficient as in the control cells. FIG. 6B (40X magnification) shows that FH535 did not disrupt normal interaction with the nuclear export protein CRM1. Colocalization with CRM1 can be seen at the early stages of infection. Cells were stained with antibodies targeting the virus NP as well as the nuclear export protein CRM1. At each time point measured, there was a clear difference in the number of infected cells in the FH535-treated cells compared to controls (FIG. 6A). Compound FH535 did not disrupt normal interactions between virus nucleoprotein (NP) and CRM1 (FIG. 6B) demonstrating that nuclear export was not affected.

Example 13

Infected cells harvested at 16, 20 and 42 hours were used for Western blot analysis to confirm apparent down regulation of the IAV related protein NP. FIG. 7 shows A549 cells that were infected at an MOI of 10 with influenza A strain PR8 and cells were harvested at several time points. Protein extracts were analysed by Western blot for virus NP to verify microscopy results which showed a significant decrease in virus nucleoprotein (NP) in cells treated with 3 μ M of FH535. C = control, T = treated. Samples were normalised to GAPDH. When compared to the control infections, FH535-treated cells had reduced NP protein up to the late stages of infection (FIG. 7) suggesting that inhibition may have occurred at an early stage and remains constant during the course of the infection.

Taken together, the Examples may demonstrate that the Wnt/ β -Catenin pathway regulates IAV replication in these *in vitro* assays. The Examples also demonstrate a

decrease in virus titres in IAV infected cells that were treated with the bioactive compound FH535, a known inhibitor of the Wnt/ β -Catenin signaling pathway.

It is known that *in vitro* data is often used as a basis for further *in vivo* studies and applications and clinical applications in human subjects. In particular, in the field of

5 Wnt/ β -Catenin signaling pathway, *in vitro* studies commonly form the basis of further *in vivo* and clinical applications. For example, Lui et al. (2016) *FH535, A β -Catenin pathway inhibitor, represses pancreatic cancer xenograft growth and angiogenesis* Oncotarget, Vo. 7, No. 30 showed that FH535 acts as a Wnt/b-catenin pathway inhibitor to inhibit pancreatic cancer cell growth *in vitro*. Those authors also confirm that this

10 bioactive compound may also work in a mouse xenograft model.

CLAIMS

1. Use of a bioactive compound as an inhibitor or a downregulator of a Wnt/ β -Catenin signalling pathway for inhibiting replication of Influenza A virus in a cell.
2. The use of claim 1, wherein the bioactive compound is selected from a group consisting of FH535; XAV939; IWP2; IWR1; IWP12; IWP-2; JW74; JW55; Okadaic acid; Tautomycin; SB2390963; SB203580; 2-[4-(4-fluorophenyl)piperazin-1-yl]-6-methylpyrimidin-4(3H)-one; PJ34; Nicosamide; Cambinol; Sulindac; 3289-8625; Scaffold A for a series of analogues; Scaffold B for a series of analogues; J01-017a; NSC668036; Filipin; IC261; PF670462; Bosutinib; PHA665752; Imatinib; ICG-001; Ethacrynic acid derivatives; PKF115-584; PNUI-74654; PKF118-744; CGP049090; PKF118-310; ZTM000990; BC21; GDC-04; and Rp-8-Br-cAMP.
3. The use of claim 2 wherein the bioactive compound is FH535.
4. The use of claim 1 wherein the bioactive compound inhibits or downregulates aspects of the Wnt/ β -Catenin signalling pathway selected from a group consisting of: TCF/LEF transcription factors; receptors for TCF/LEF transcription factors; Tankyrase 1; Porcupine (PORCN); Tankyrase 2; PP2A phosphate; PP1 phosphatase; p38 MAPK; Dvl-2; SIRT1; a PDZ domain of Dishevelled; caveolin-mediated endocytosis; CK1 ϵ ; CK1 δ ; Src kinase; c-Met; CREB binding protein; Lef-1; β -Catenin; and PKA.
5. The use of claim 4 wherein the bioactive compound inhibits or downregulates the TCF/LEF transcription factors or alternatively, receptors for the TCF/LEF transcription factors.

6. A medicament for inhibiting replication of Influenza A virus, the medicament comprising:
 - (a) a bioactive compound that inhibits the Wnt/ β -Catenin signalling pathway; and
 - (b) one or more pharmaceutically acceptable excipients.
7. The medicament of claim 6 wherein the bioactive compound is selected from a group consisting of FH535; XAV939; IWP2; IWR1; IWP12; IWP-2; JW74; JW55; Okadaic acid; Tautomycin; SB2390963; SB203580; 2-[4-(4-fluorophenyl)piperazin-1-yl]-6-methylpyrimidin-4(3H)-one; PJ34; Nicosamide; Cambinol; Sulindac; 3289-8625; Scaffold A for a series of analogues; Scaffold B for a series of analogues; J01-017a; NSC668036; Filipin; IC261; PF670462; Bosutinib; PHA665752; Imatinib; ICG-001; Ethacrynic acid derivatives; PKF115-584; PNUI-74654; PKF118-744; CGP049090; PKF118-310; ZTM000990; BC21; GDC-04; and Rp-8-Br-cAMP.
8. The medicament of claim 7 wherein the bioactive compound is FH535.
9. The medicament of claim 6 wherein the bioactive compound inhibits or downregulates an aspect of the Wnt/ β -Catenin signalling pathway that is selected from a group consisting of: TCF/LEF transcription factors; receptors for TCF/LEF transcription factors; Tankyrase 1; Porcupine (PORCN); Tankyrase 2; PP2A phosphate; PP1 phosphatase; p38 MAPK; Dvl-2; SIRT1; a PDZ domain of Dishevelled; caveolin-mediated endocytosis; CK1 ϵ ; CK1 δ ; Src kinase; c-Met; CREB binding protein; Lef-1; β -Catenin; and PKA.
10. The medicament of claim 6 wherein the bioactive compound inhibits or downregulates the TCF/LEF transcription factors or receptors for the TCF/LEF transcription factors.
11. The medicament of any one of claims 6 to 10, wherein the one or more pharmaceutically acceptable excipients are useful for administering the medicament by a route selected from a group consisting of: oral administration;

intravenous injection; intramuscular injection; sub-cutaneous injection; intrathecal injection; sublingual placement; buccal placement; rectal insertion; vaginal insertion; ocular placement; otic placement; topical cutaneous application; topical nasal application; topical oral application; oral inhalation; nasal inhalation; transdermal application or combinations thereof.

12. The medicament of any one of claims 6 to 10, wherein the medicament is nebulized.
13. A method of treating an Influenza A viral infection comprising;
administering to a subject in need thereof, a composition comprising a bioactive compound and one or more pharmaceutically acceptable excipients, wherein the bioactive compound inhibits the Wnt/ β -Catenin signalling pathway.
14. The method of claim 13 wherein the subject has a predisposition to IAV infection, and the step of administering is a prophylactic administration.
15. The method of claim 13 wherein the subject demonstrates IAV infection indications, and the step of administering is a reactive administration.

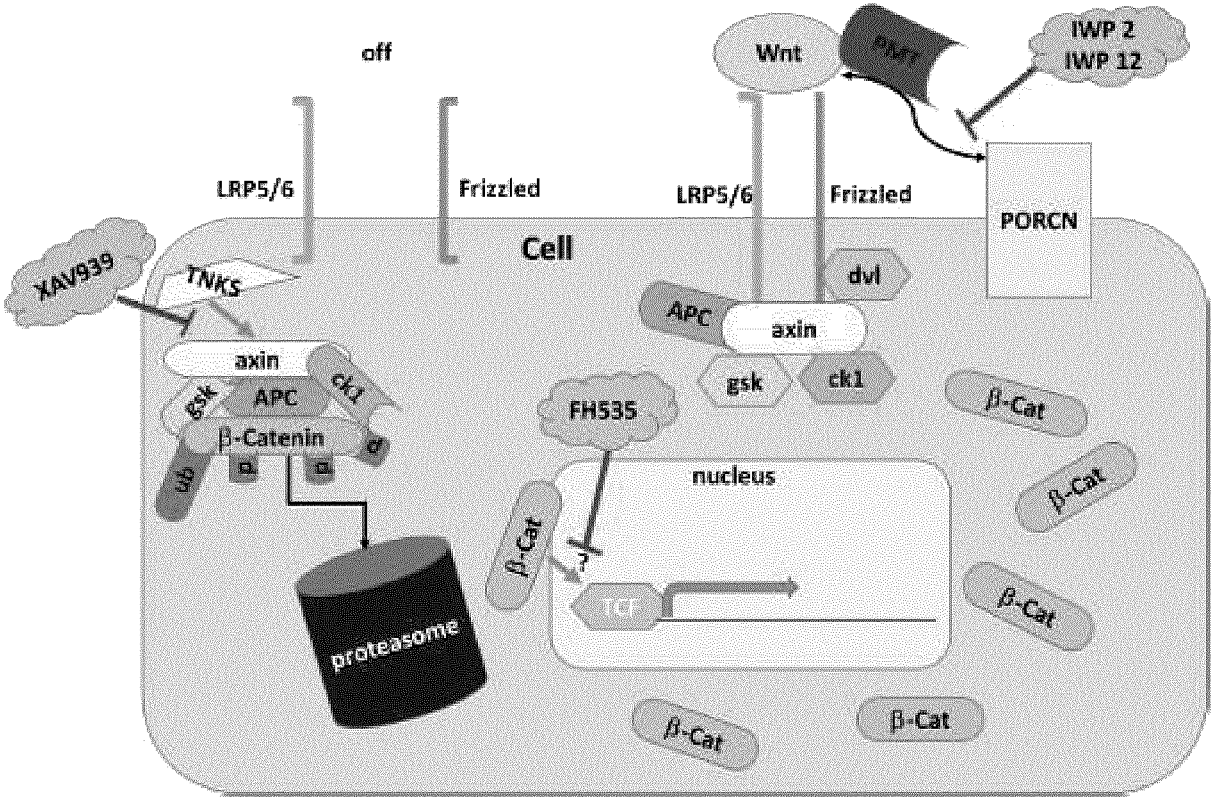


FIG. 1

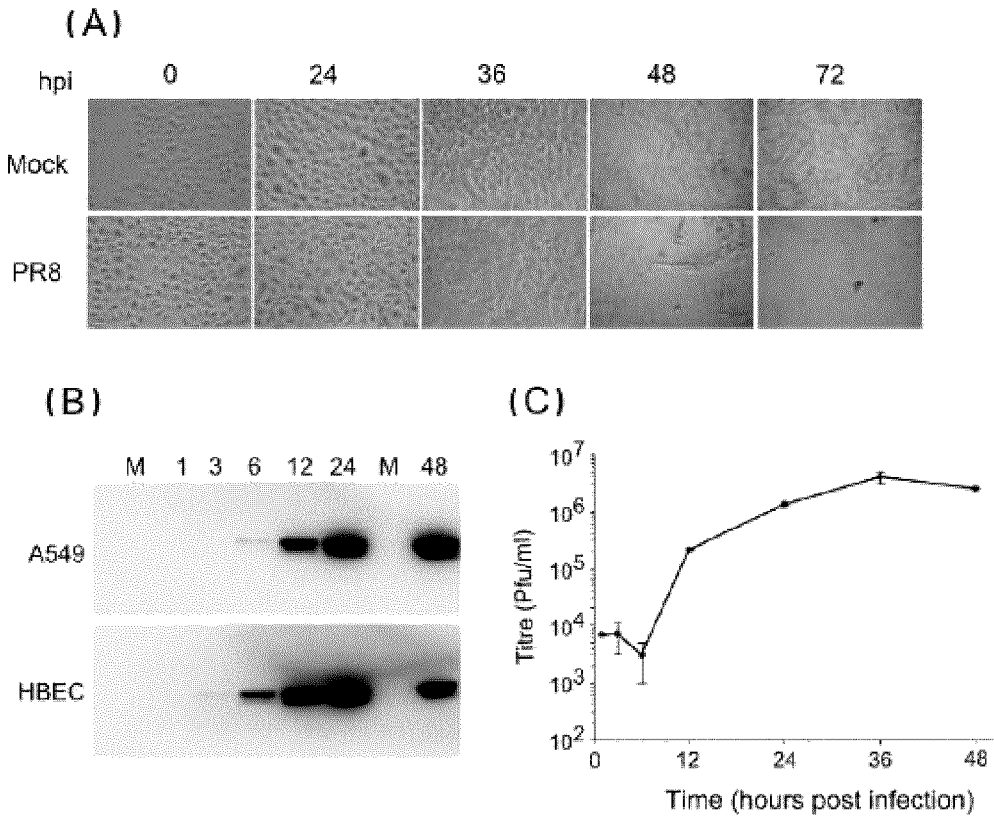


FIG. 2

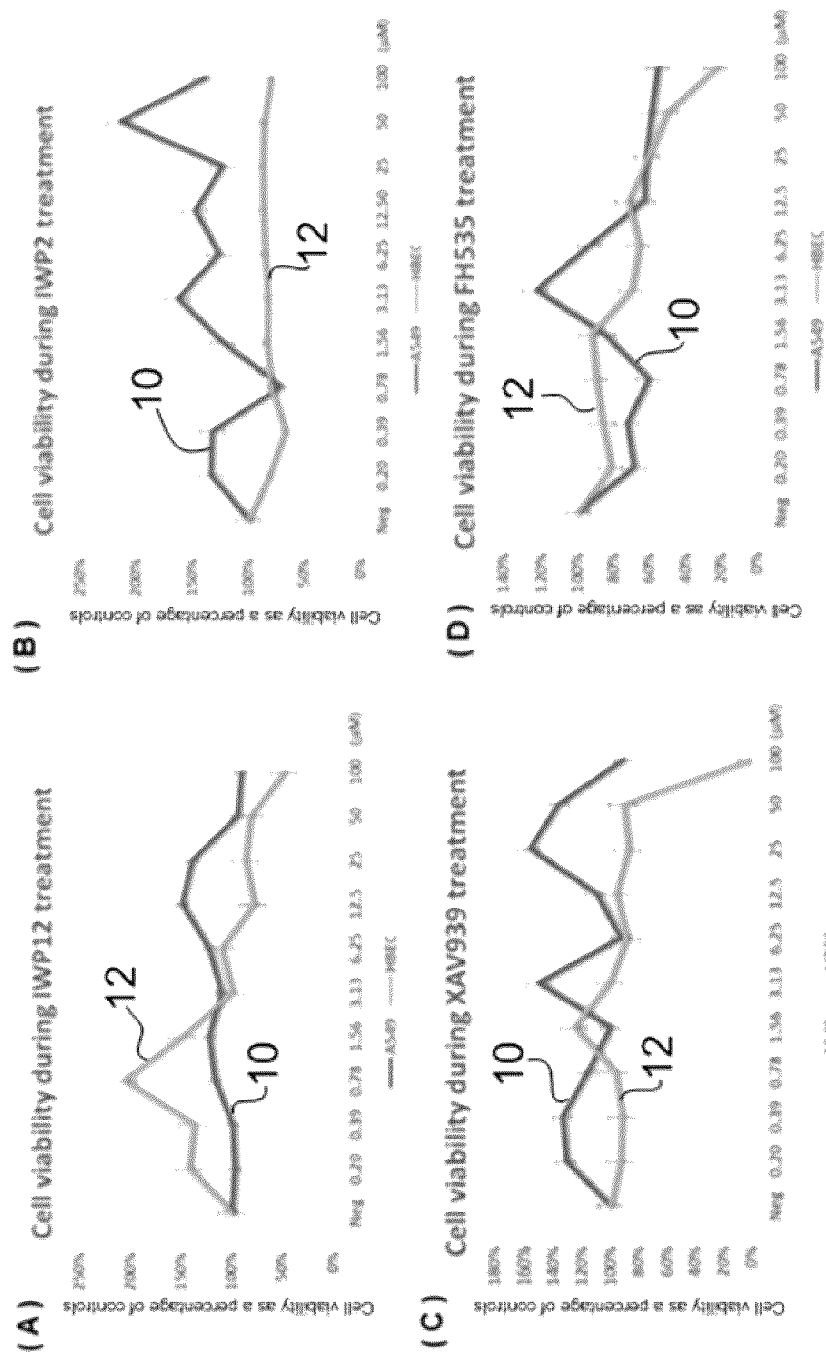


FIG. 3

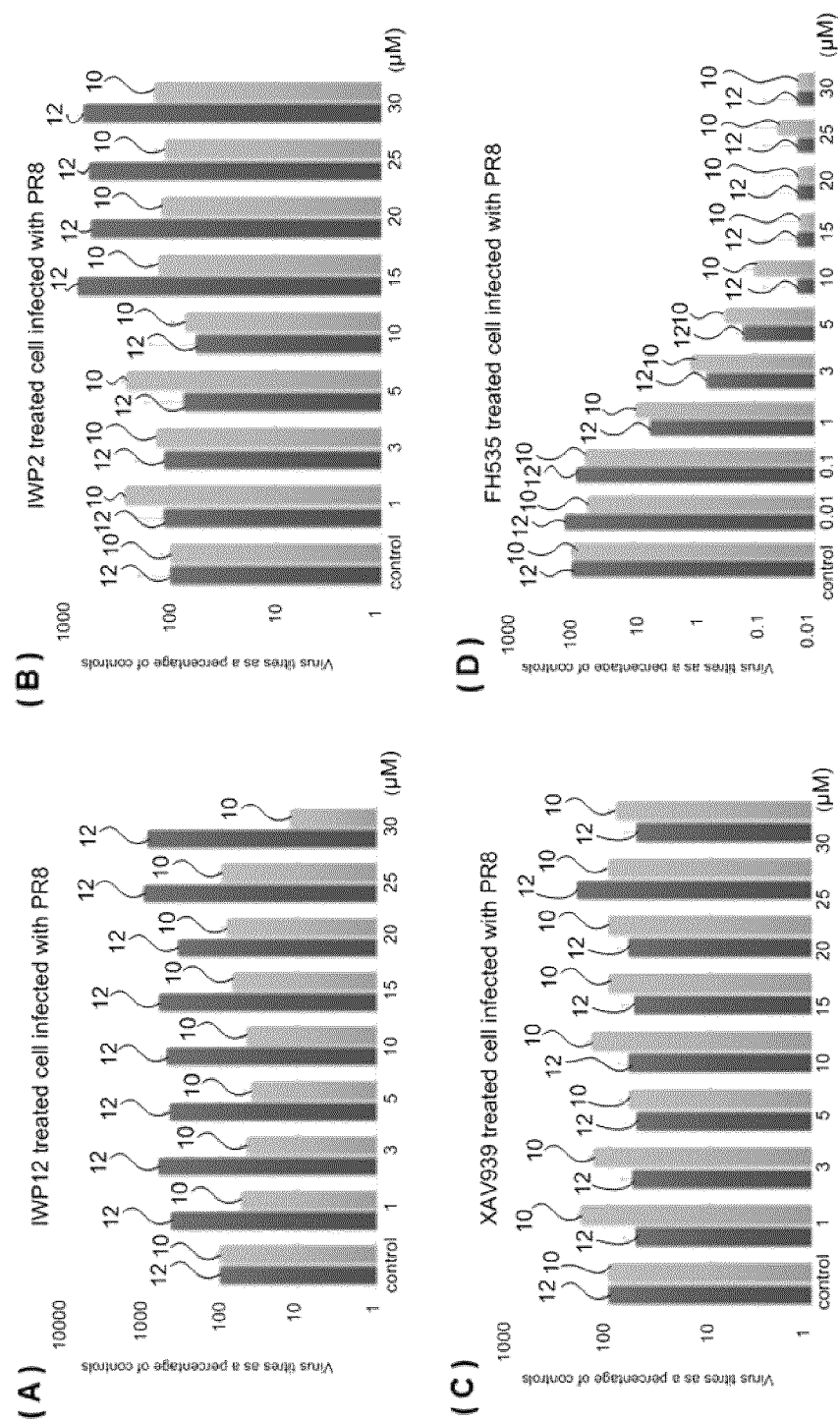


FIG. 4

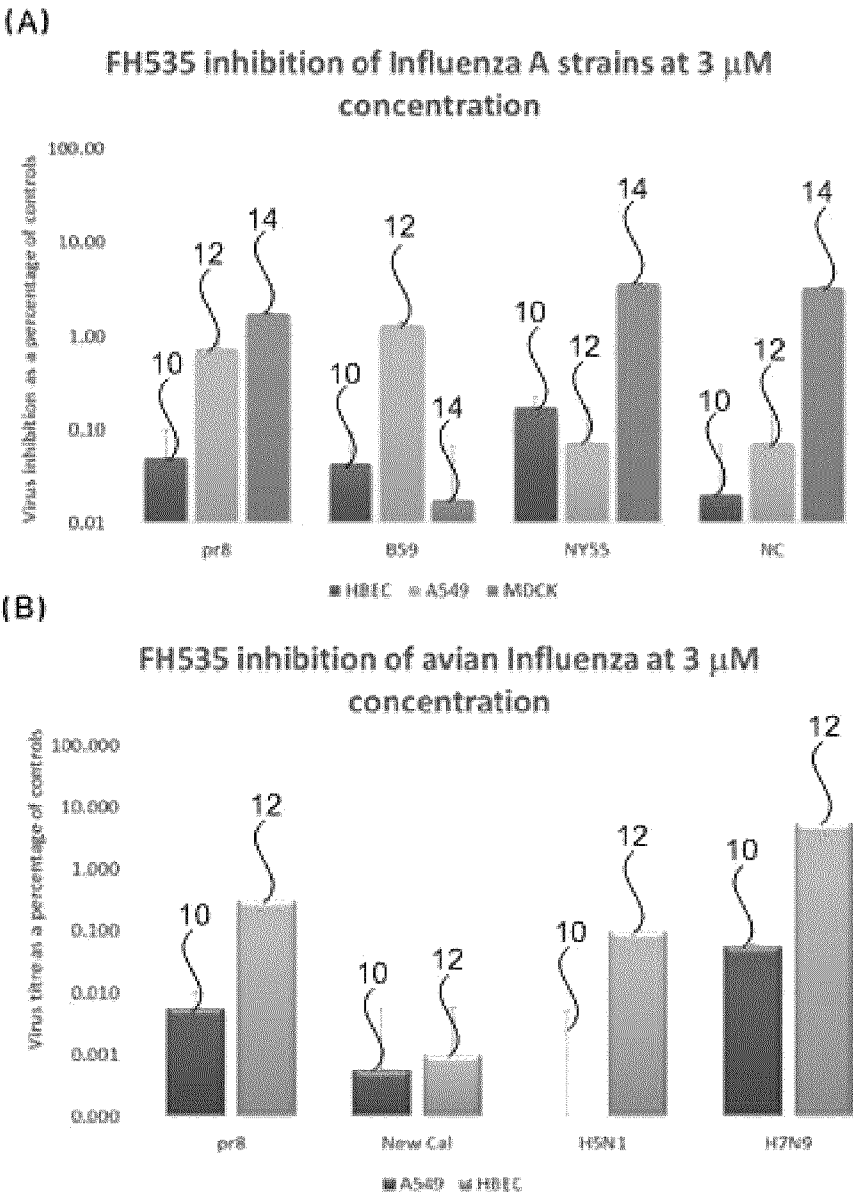


FIG. 5

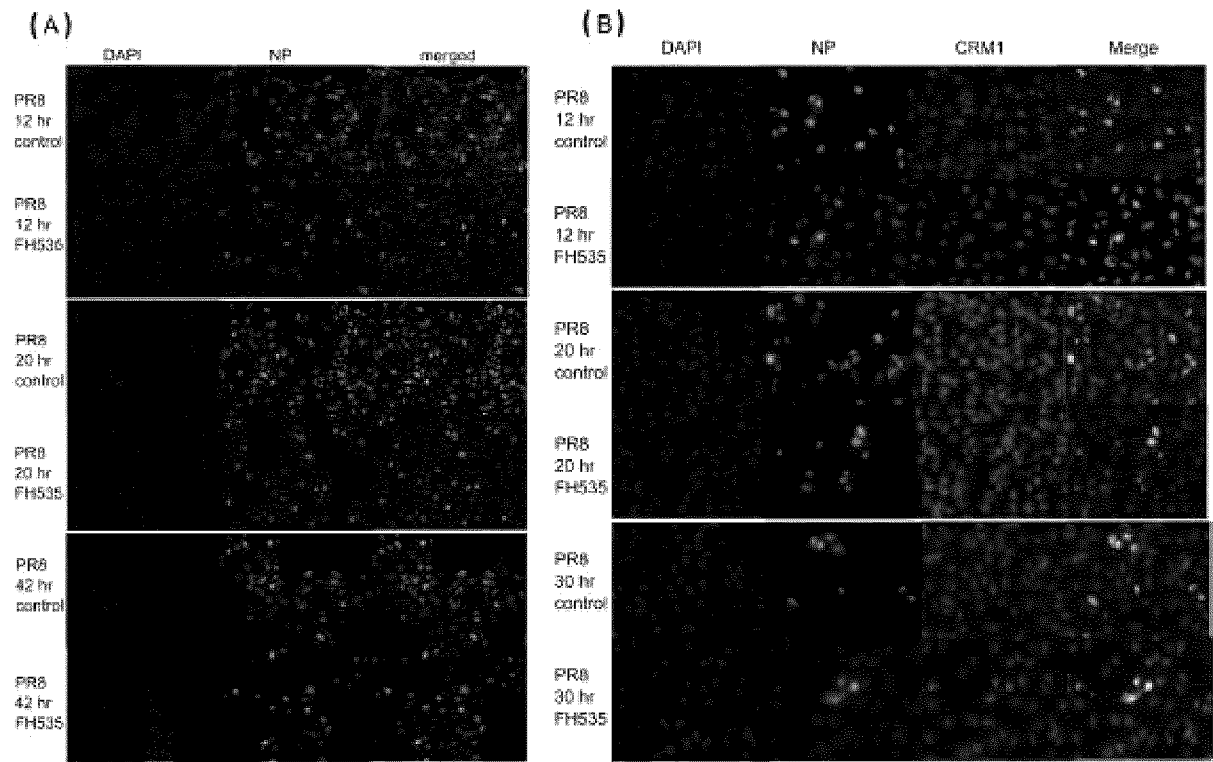


FIG. 6

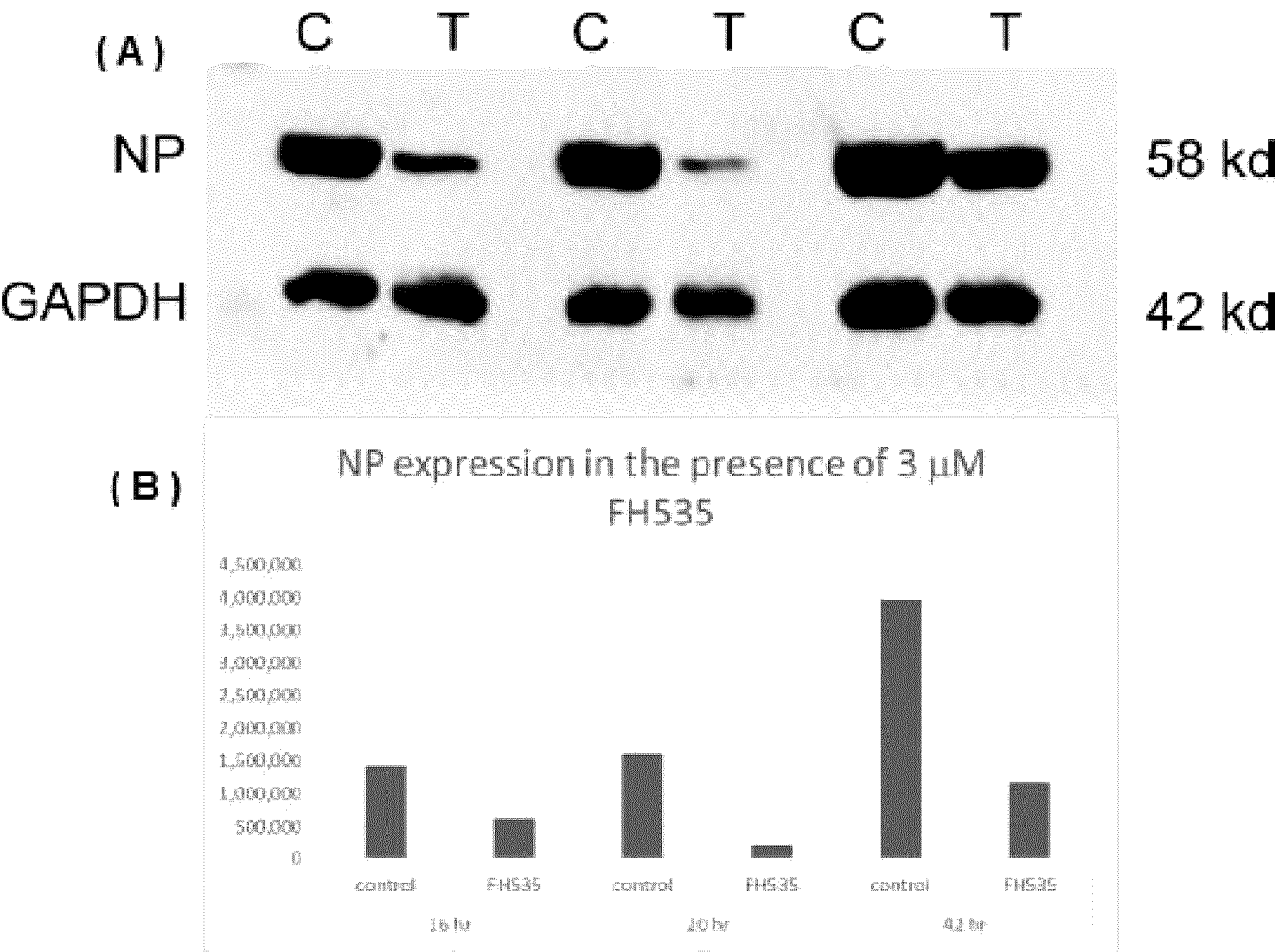


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/051339

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 31/18** (2006.01), **A61K 9/72** (2006.01), **A61P 31/14** (2006.01), **C07C 311/21** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K (2006.01), A61P (2006.01), C07C (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Canadian Patent Database, Questel Orbit, Scopus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/134525 A1 (LIU et AL) 11 September 2015 (11-09-2015) (abstract, page 11-13, claims 8, 11, 12)	1, 2, 4-7, 9-15
X	US 2006/0275260 A1 (RIVIERE et AL) 7 December 2006 (07-12-2006) (abstract)	1, 2, 4-7, 9-15
X	WO 2012/106509 A1 (SHENK et AL) 9 August 2012 (09-08-2012) (Figure 9)	1, 2, 4-7, 9-15
X	WO 2011/088474 A2 (MCDONALD et AL) 21 July 2011 (21-07-2011) (whole document)	1, 2, 4-7, 9-15

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
24 January 2017 (24-01-2017)Date of mailing of the international search report
23 February 2017 (23-02-2017)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Orysia Zaporozan (819) 639-9424

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/051339**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 13-15
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 13-15 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 13-15.

2. ☒ Claim Nos.: 1, 2, 4-7, 9-15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See supplemental box

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See supplemental box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

- Remark on Protest**
- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/051339

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2425837 (JIANG CHENGYU et AL) 7 March 2012 (07-03-2012) (whole document)	1, 2, 4-7, 9-15
A	HANDELI et al., " <i>A small-molecule inhibitor of Tcf/β-catenin signaling down-regulates PPARγ and PPARδ activities</i> ". Mol Cancer Ther, March 2008 (03-2008), Vol. 7(3), pp. 521-9, (whole document)	1-15

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/051339

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2015134525A1	11 September 2015 (11-09-2015)	None	
US2006275260A1	07 December 2006 (07-12-2006)	US2006275260A1	07 December 2006 (07-12-2006)
		US8293745B2	23 October 2012 (23-10-2012)
		AP200603863D0	31 December 2006 (31-12-2006)
		AP2715A	31 July 2013 (31-07-2013)
		AR049141A1	28 June 2006 (28-06-2006)
		AU2005249159A1	15 December 2005 (15-12-2005)
		BRPI0511805A	15 January 2008 (15-01-2008)
		CA2568671A1	15 December 2005 (15-12-2005)
		CN1976709A	06 June 2007 (06-06-2007)
		CN102085202A	08 June 2011 (08-06-2011)
		DK1750713T3	11 February 2013 (11-02-2013)
		EA200602289A1	27 April 2007 (27-04-2007)
		EA013813B1	30 August 2010 (30-08-2010)
		ECSP055833A	20 September 2005 (20-09-2005)
		EP1750713A1	14 February 2007 (14-02-2007)
		EP1750713A4	15 September 2010 (15-09-2010)
		EP1750713B1	17 October 2012 (17-10-2012)
		ES2397830T3	11 March 2013 (11-03-2013)
		IL179796D0	15 May 2007 (15-05-2007)
		JP2008501646A	24 January 2008 (24-01-2008)
		JP5015769B2	29 August 2012 (29-08-2012)
		KR20070051832A	18 May 2007 (18-05-2007)
		KR101246863B1	25 March 2013 (25-03-2013)
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		MY148676A	31 May 2013 (31-05-2013)
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		PT1750713E	25 January 2013 (25-01-2013)
		TWI393565B	21 April 2013 (21-04-2013)
		UY28942A1	30 November 2005 (30-11-2005)
		WO2005117885A1	15 December 2005 (15-12-2005)
WO2012106509A1	09 August 2012 (09-08-2012)	WO2012106509A1	09 August 2012 (09-08-2012)
		CN103391777A	13 November 2013 (13-11-2013)
		EP2670404A1	11 December 2013 (11-12-2013)
		JP2014505705A	06 March 2014 (06-03-2014)
		US2013338178A1	19 December 2013 (19-12-2013)
		US2016296523A1	13 October 2016 (13-10-2016)
WO2011088474A2	21 July 2011 (21-07-2011)	WO2011088474A2	21 July 2011 (21-07-2011)
		WO2011088474A3	29 December 2011 (29-12-2011)
EP2425837A1	07 March 2012 (07-03-2012)	EP2425837A1	07 March 2012 (07-03-2012)
		EP2425837A4	23 January 2013 (23-01-2013)
		EP2425837B1	04 March 2015 (04-03-2015)
		CN101869568A	27 October 2010 (27-10-2010)
		DK2425837T3	26 May 2015 (26-05-2015)
		JP2012530685A	06 December 2012 (06-12-2012)
		JP5658741B2	28 January 2015 (28-01-2015)
		US2012238566A1	20 September 2012 (20-09-2012)
		WO2010124618A1	04 November 2010 (04-11-2010)

Continued from Box II

The International Searching Authority has not carried out a search for claims 1, 2, 4-7, 9-15, under Article 17(2)(b) of the PCT. The description, claims, and/or drawings fail to comply with the prescribed requirements to such an extent that a meaningful search could not be carried out. Claims 1, 2, 4-7, 9-15 so lack clarity and/or support that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been established for the parts of the application which appear to be clear and supported, namely the compounds defined in claims 2 and 7 with the exception of "scaffold A for a series of analogues", "scaffold B for a series of analogues" and "SB2390963" since these compounds were not adequately defined.

Continued from Box III

The claims are directed to a plurality of inventive concepts as follows:

- Group A - Claims 3, 8; 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting replication of Influenza A virus in a cell, wherein the bioactive compound is FH535;
- Group B - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting replication of Influenza A virus in a cell, wherein the bioactive compound is XAV939;
- Group C - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting replication of Influenza A virus in a cell, wherein the bioactive compound is IWP2;
- Group D - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting replication of Influenza A virus in a cell, wherein the bioactive compound is IWR1;
- Group E - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is IWP12;
- Group F - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is JW74;
- Group G - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is JW55;
- Group H - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is okadaic acid.;
- Group I - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is tautomycin.;
- Group J - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is SB2390963.;
- Group K - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signalling pathway for inhibiting, wherein the bioactive compound is SB203580.;
- Group L - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is 2-[4-4-fluoro-phenylpiperazin-1-yl]-6-methylpyrimidin-4(3H)-one.;
- Group M - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PJ34.;
- Group N - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is nicosamide.;
- Group O - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is cambinol.;
- Group P - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is sulindac.;
- Group Q - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is 3289-8625.;
- Group R - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is "scaffold A for a series of analogues".;
- Group S - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is "scaffold B for a series of analogues".;
- Group T - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is J01-017a.;
- Group U - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is NSC668036.;
- Group V - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is filipin.;
- Group W - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is IC261.;

Group X - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PF670462.;

Group Y - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is bosutinib.;

Group Z - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PHA665752.;

Group AA - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is imatinib.;

Group BB - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is ICG-001.;

Group CC - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is ethacrynic acid derivatives.;

Group DD - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PFK115-584.;

Group EE - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PNU1-74654.;

Group FF - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PKF118-744.;

Group GG - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is CGP049090.;

Group HH - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is PKF118-310.;

Group II - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is ZTM000990.;

Group JJ - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is BC21.;

Group KK - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is GDC-04.;

Group LL - Claims 1, 2, 4-7, 9-15 (in part) are directed to the use of a bioactive compound as an inhibitor or downregulator of a Wnt/ β -Catenin signaling pathway for inhibiting, wherein the bioactive compound is Rp-8-Br-cAMP.